

Immunotherapy with Alpare® in patients with respiratory allergy to *Parietaria* pollen: a two year double-blind placebo-controlled study

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Summary

Allergy to *Parietaria judaica* pollen causes significant morbidity in many areas of the world. In addition to rhinitis, patients who are allergic to this pollen have a high incidence of asthma. The pollinating season is long, making this particular allergy challenging for clinicians to treat. This study was designed to determine if immunotherapy with an alum adsorbed partially purified *Parietaria* extract (Alpare *Parietaria*) containing a targeted maintenance dose of 12 500 BUs was effective in decreasing rhinitis symptoms in patients allergic to *Parietaria*. Using a double-blind placebo-controlled technique 36 patients received placebo or active extract for 2 years. Twenty (11 placebo and nine active) completed the 2 year study. Efficacy of treatment was evaluated by determining changes in skin reactivity, visual analog scores, diary symptom scores and end of study assessments. Reactions were monitored as well. Skin-test suppression was marginally significant in the actively treated group after 1 year and showed even more significant suppression after the second year. Nasal block, rhinorrhoea and sneezing all were significantly decreased in the active group. The nasal provocation test did not show a significant change after 1 year, in either group, but after 2 years of treatment the active group did show significant improvement. Although almost all patients in the actively treated group experienced local reactions, the incidence of systemic reactions was not different between the two groups. In conclusion, immunotherapy with this extract at this dose was effective in ameliorated rhinitis symptoms in patients allergic to *Parietaria judaica*.

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Introduction

It is generally accepted that specific immunotherapy (SIT) of IgE mediated respiratory disorders is efficacious when administered under appropriate circumstances. Its efficacy has been confirmed in a limited number of carefully controlled clinical studies [1–8]. Controversy still exists about optimum extract doses, immunotherapy schedules, and the use of different types of extracts [9–14].

Parietaria judaica pollen is a common cause of allergy in the Mediterranean area and in particular in the Naples

area [15–19]. Because of its long pollinating season and high levels of pollination, sensitive patients experience symptoms which can be quite severe and difficult for clinicians to treat.

While several controlled studies have demonstrated the efficacy of SIT in pollen allergy [20–23], we were able to identify only two double-blind placebo-controlled studies involving SIT of *Parietaria* induced rhinitis. The first involved subcutaneous injections of an alginate-conjugated extract [24]. The second used nasal inhalations of an extract in macronized powder form [25]. Because demonstrations of efficacy for one allergen or one allergenic extract do not necessarily mean that efficacy will occur with other allergens or extracts there is a clear need

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for carefully controlled studies demonstrating such efficacy. Studies using challenge techniques are particularly valuable in demonstrating efficacy of SIT [26–30].

Alpare is a partially purified (dialysed) alum adsorbed extract that is standardized by skin-testing sensitive patients and comparing reactivity to that obtained from a 10 mg/ml histamine dihydrochloride control. Potency is expressed in AURs/ml [31]. Patients in this study were treated for 2 years with a maximum targeted dose of 80 AURs. This is equivalent to 12 500 BUs as defined in the Nordic Guidelines [32].

The objectives of this study were to assess the clinical efficacy and safety of Alpare *Parietaria* relative to placebo in patients with respiratory allergy to *Parietaria judaica* pollen. We monitored effectiveness for rhinitis by symptom scores, nasal challenges, skin-test reactivity and patient diaries.

Methods and materials

Subjects

Thirty-six subjects were enrolled in the study. To be included subjects had a history of rhinitis with or without asthma during the *Parietaria* pollen season. They also exhibited a positive skin-prick test and nasal provocation test to *Parietaria* extract, as well as a RAST score of 2 or greater to *Parietaria*. At entry, one patient exhibited a RAST score of 2 to *Parietaria*, all others had scores of 3 or 4. In addition, they exhibited negative skin-prick tests to Timothy and mite extracts. Subjects were not to have received immunotherapy with any *Parietaria* extract within the previous 3 years. Subject characteristics are listed in Table 1. Since only seven of the subjects had asthma in addition to rhinitis we have limited our analysis to the data concerned with rhinitis. More patients in the active group had asthma than in the

placebo group. The asthma in these patients was mild and did not require medication which may have influenced our rhinitis assessment.

Study design

Prior to initiation of therapy in the autumn of 1990, the following baseline data were collected: skin-prick tests with five concentrations of Alphabase *Parietaria*, nasal provocation test with Alphabase *Parietaria*, subjective assessment of severity of symptoms during the 1990 pollen season and a nasal symptom visual analog scale. This same data was collected after 12 and 24 months of therapy. In addition, during the pollen seasons of 1991 and 1992 each patient kept a daily symptom diary.

Skin-prick test

Fresh dilutions for skin testing purposes were made the day of testing. Skin-prick tests were performed using DHS Lanceters; 1000, 200, 40, 8 and 1.6 AURs/ml in duplicate, one on each arm, were tested. The weals were outlined and measured by planimetry. The mean of the two measurements was used for data analysis.

Nasal provocation test

Parietaria Alphabase, a sterilized form of lyophilized Alpha fraction extract, was reconstituted in normal saline to give a stock solution of 1000 AUR/ml. Fivefold serial dilutions were prepared. One drop (0.05 ml) of saline was placed in each nostril as a control. If no symptoms occurred in 15 min, 0.05 ml of the weakest concentration of the test material was placed in each nostril. If no response occurred in 15 min the next most concentrated solution was administered. A response was defined as positive if there were at least three of the

Table 1. Patient characteristics

	At enrolment		After 12 months		After 24 months	
	Placebo	Active	Placebo	Active	Placebo	Active
Number of subjects	17	19	15	13	11	9
Age range	20–67	15–47	20–67	15–47	20–67	20–47
(Mean)	(34.6)	(28.6)	(34.2)	(29.5)	(33.8)	(31.8)
Sex	10M/7F	10M/9F	9M/6F	6M/7F	6M/5F	5M/4F
Rhinitis only	15	9	13	6	10	3
Rhinitis & asthma	2	10	2	7	1	6
SPT range (Adm)	18–85	19–105	18–85	19–105	18–85	19–105
(Mean)	(48)	(41)	(42)	(43)	(43)	(45)

following: sneezing, itching, increased nasal secretions, increased nasal congestion, increased oedema, or increased nasal pallor.

Diaries

Patients were asked to keep a diary listing their symptoms during the *Parietaria* pollen seasons in 1991 and 1992. Patients were asked to rate their symptoms from 0 (no symptoms) to 3 (severe symptoms) daily during these time periods. Medication use was also listed in the diaries.

Reactions

Patients were asked to remain in the clinic for at least 30 min after each injection. Before receiving an injection they were questioned regarding local and systemic reactions after the previous injection. If such reactions occurred, they were described in the case report form.

Allergenic extracts

Skin tests were performed using Dome/Hollister-Stier (DHS) extracts. On admission subjects were skin-prick tested to Alphatest *Parietaria* 400 AUR/ml, Alphatest Timothy 400 AUR/ml, Alphatest *D. pteronyssinus* 400 AUR/ml, histamine 10 mg/ml and glycerococaine's solution. Skin tests were performed using lyophilized Alphasbase *Parietaria* reconstituted in glycerococaine's solution to give concentrations of 1.6, 8, 40, 200 and 1000 AURs/ml. *Alpare Parietaria* treatment sets or placebo consisting of aluminum hydroxide suspension (in same concentrations as in *Alpare Parietaria*) with 5 µg histamine dihydrochloride per ml were administered. All patients were able to tolerate 0.8 cc of the top strength of active extract or placebo. During the *Parietaria* pollen season the maintenance doses were decreased in all patients.

Visual analog scores (VAS)

Subjects were asked to estimate the severity of symptoms by marking a scale 10 cm in length. One end (0) of the scale was labelled 'no symptoms'. The other end (10) of the scale was labelled 'symptoms could not be worse'. The length of the response was measured and recorded at the appropriate time points.

Pollen counts

Pollen counts were performed with a VPPS-2000 7-day recording volumetric spore trap. The trap was placed on the top of the hospital, approximately 25 m above the

ground. The atmospheric presence of *Parietaria* pollen grains was expressed as daily mean concentration/m³ of air.

Statistical methodology

All analyses for efficacy variables include only those patients who completed the 2 year study. Analysis of injection reactions include all available data from patients who participated in the study.

Skin-prick tests results were analysed for each year with a repeated measures model that included the terms for the effect of the treatment group, subject within treatment group, concentration level, and treatment group by concentration interaction. Because the data representing the absolute reaction size was not considered to be normally distributed, the following transformation was made and used as the dependent variable: $\log[(x + 1)/(base + 1)]$ where x = absolute reaction size during treatment and $base$ = absolute reaction size at baseline.

Results from the visual analogue scale were analysed with a two sample *t*-test. To adjust for differences which may have existed at baseline the analysis was conducted on the change from baseline scores. If a preliminary test indicated significantly different variances at the 0.01 level Cochran and Cox's approximation to the *P*-value of the approximate *t*-test was used with Satterthwaite's approximation to the degrees of freedom [33–35].

Individual means were calculated for each week of each patient's diary. The group weekly means for these calculations were analysed with a two sample *t*-test.

The number of patients indicating improvement in nasal provocation tests, and end of study rhinitis assessment was analysed with Fisher's exact test.

Results

Of the 36 patients who started the study, data were available for only 27 (15 placebo and 12 active patients) at the end of year 1 and only 20 (11 placebo and nine active patients) at the end of year 2.

The reasons for patient drop-outs are listed in Table 2. The drop-outs did not appear to be due to ineffective treatment or adverse effects which might have biased our efficacy or safety evaluations. One of the patients who dropped out from the active treatment group reported large local reactions.

Skin tests

We examined skin-test variability by comparing right and left arm weal measurements. In general when mean

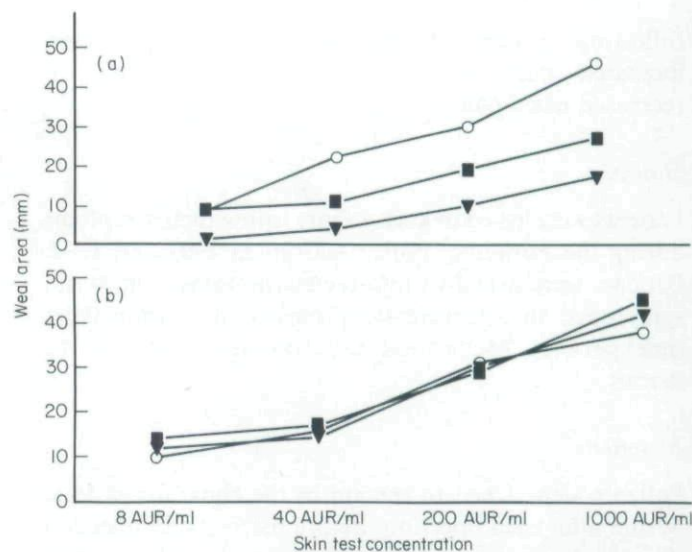
Table 2. Patient drop-outs

	Number of patients	
	Active group	Placebo group
Completed 1 year of treatment, had good results but did not return	5	0
No improvement, so discontinued therapy	1	6
Dropped out due to pregnancy	3	0
Local reactions, could not build to maintenance dose	1	0

weal responses were greater than 20 mm the mean absolute difference between arms was only around 10 mm (range 8.0–11.2 mm).

As shown in Fig. 1 patients receiving placebo indicated little change from baseline at any concentration during the study.

With only one exception (year 1, 8 AUR/ml) the mean size of skin-test reactions for patients in the Alpare

**Fig. 1.** Skin-test results. (a) Active group. (b) Placebo group. —○—. Baseline. —■—. Year 1. —▼—. Year 2.

Parietaria group was less after treatment than at baseline. In general, the amount of improvement increased with greater concentrations and was greater after the second year than after the first.

The comparison of treatment groups over all concen-

Table 3. Visual analog scores for patients completing 2 years of the study

Time point	Placebo <i>n</i> = 11		Active <i>n</i> = 9	
	Mean	SD	Mean	SD
<i>Nasal block</i>				
Baseline	78.5	6.0	77.2	8.3
Year 1	77.8	5.4	29.6	20.7
Change	-0.7	8.6	-47.6*	17.2
Year 2	78.5	5.6	24.6	18.4
Change	0	7.6	-52.6*	15.9
<i>Rhinorrhoea</i>				
Baseline	71.0	9.8	70.4	8.9
Year 1	71.5	7.1	26.0	19.6
Change	0.5	13.1	-44.4*	13.6
Year 2	77.5	6.2	27.8	19.5
Change	6.5	10.3	-42.6*	13.4
<i>Sneezing</i>				
Baseline	76.5	8.2	68.7	12.0
Year 1	73.4	5.4	29.4	21.4
Change	-3.1	8.9	-39.3*	19.6
Year 2	74.5	7.5	25.1	19.4
Change	-2.0	10.6	-43.6*	14.2

* Statistically significantly different from placebo at $P < 0.01$ level.

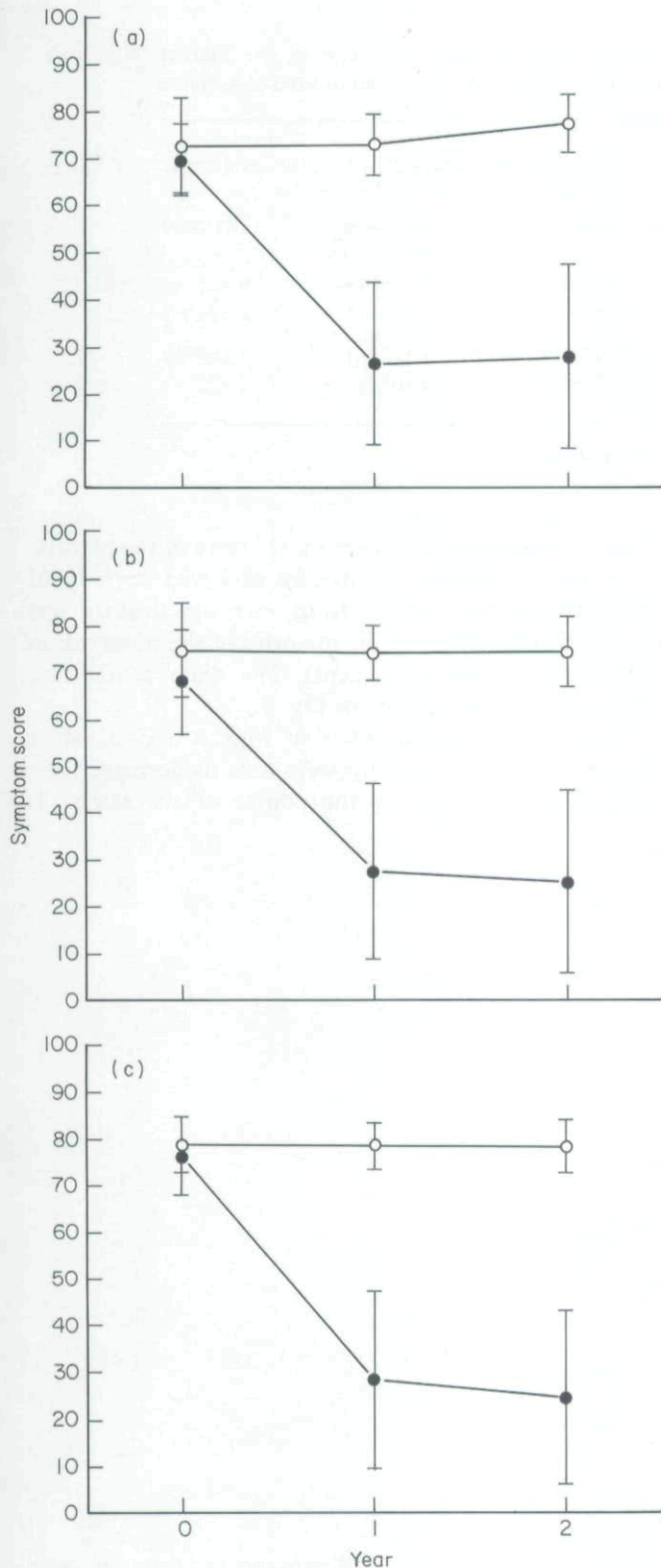


Fig. 2. Visual analog scores (mean and standard deviation) for (a) Rhinorrhoea. (b) Sneezing. (c) Nasal block. ○. Placebo. ●. Active.

treatments after 1 year of treatment was marginally significant ($P = 0.05$). The mean reaction had decreased 11.4 mm and 19.1 mm at the 40 and 1000 AUR/ml concentrations, respectively. Both these decreases were significantly ($P < 0.05$) greater than those observed at the same concentrations in the placebo group.

The comparison of treatment groups over all concentrations after 2 years of treatment was statistically significant ($P < 0.01$). The mean reaction had decreased 7.2, 18.4, 20.1, and 28.8 mm, relative to baseline, at the 8, 40, 200, and 1000 AUR/ml concentrations, respectively. Each of these decreases was significantly ($P < 0.01$) greater than those observed at the same concentrations in the placebo group.

VAS scores

As shown in Table 3 and Fig. 2, patients rated the symptoms of nasal block, rhinorrhoea, and sneezing before and after 1 and 2 years of treatment.

In general, patients in the placebo group indicated little change from baseline in any of the symptoms measured by the VAS. Patients in the active treatment group indicated a mean improvement from baseline in all symptoms evaluated by the VAS. Most notable were the improvements in the most severe symptoms. Patients in the active treatment group indicated a mean decrease from 77.2 to 29.6 (62%) after year 1 and from 77.2 to 24.6 (68%) after year 2 in nasal blockage. These same patients indicated a mean improvement from 70.4 at baseline to 26.0 (63%) after year 1 and from 70.4 to 27.8 (61%) after year 2 in rhinorrhoea. They also indicated a mean decrease from 68.7 to 29.4 (57%) after year 1 and from 68.7 to 25.1 (64%) after year 2 in sneezing. All these improvements were significantly ($P < 0.01$) greater than those indicated by the placebo group.

Nasal provocation test

As indicated in Table 4, relative to tests performed at baseline, six of 11 (55%) placebo patients worsened (concentration required to induce a positive reaction was less than at baseline) and three (27%) improved (concentration was greater than at baseline) after 1 year of treatment. Two (22%) *Alpare Parietaria* patients worsened while four (44%) improved. There was no statistically significant differences between the two groups in the number of patients who indicated improvement, relative to baseline, at year 1.

After 2 years of treatment significantly more ($P < 0.01$) *Alpare Parietaria* patients (seven of nine, 78%) indicated improvement, relative to baseline, than did placebo patients (one of 11, 9%).

Table 4. Nasal provocation test

Number of patients, completing 24 months of treatment, who showed a change in the extract concentration which induced a positive reaction relative to the extract concentration required at baseline

After year #	Treatment group	n	Number of patients (% of treatment group)		
			Increase	No change	Decrease
1	Placebo	11	3 (27%)	2 (18%)	6 (55%)
	Active	9	4 (44%)	3 (33%)	2 (22%)
2	Placebo	11	1 (9%)	3 (27%)	7 (64%)
	Active	9	7 (78%)*	0 (0%)	2 (22%)

* Statistically significantly different from placebo at $P < 0.01$ level.

Diaries

Patients were asked to rate their symptoms in the morning and evening on a scale from 0 (without symptoms) to 3 (severe symptoms). The summaries presented are mean symptom severities.

During the periods that diaries were kept, the mean symptom severity of patients in the *Alpare Parietaria* group was always less than observed in the placebo

group. When symptoms were most severe in the placebo group (mean severity >1.70) the observed severity of symptoms in the active group was significantly less ($P < 0.05$) (this includes the majority of the observation period that diaries were kept). The diary scores and pollen counts are depicted in Fig. 3.

Medication usage is listed in Figs 4 and 5. Most patients in the active group were able to decrease their medication usage during the course of the study. It

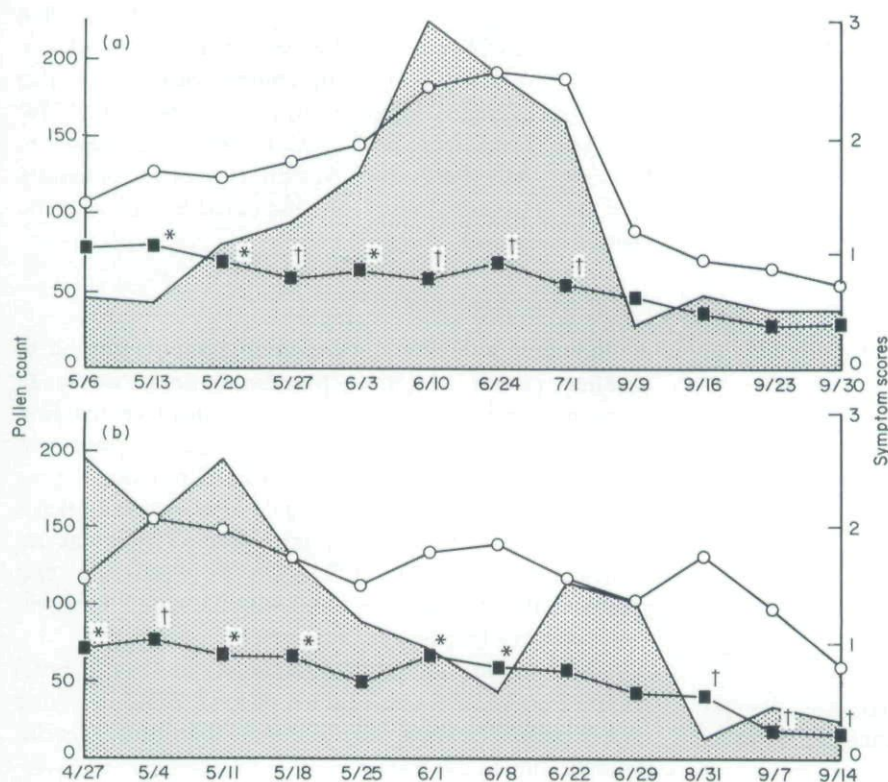


Fig. 3. Pollen counts and diary symptom scores. ■. Pollen count. ○. Placebo. ■. Active. *(†) Statistically significantly different from placebo at $P \leq 0.05$ (0.01) level. (a) 1991. (b) 1992.

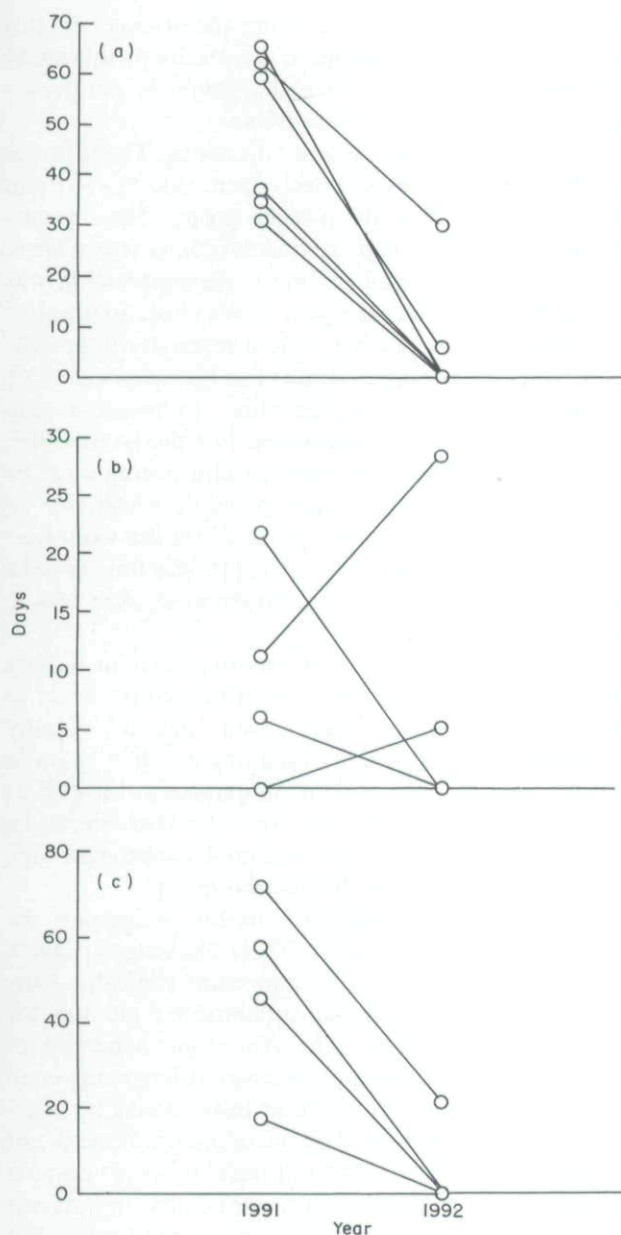


Fig. 4. Medication use for 1991 and 1992 for active patients. Results indicate days of medication use during the pollen seasons of 1991 and 1992. (a) Nasal spray. (b) Antihistamines. (c) Other medications.

did not appear that increasing medication use contributed to the efficacy observed in the active treatment group.

Reactions

Significantly ($P < 0.01$) more patients experienced some type of injection reaction during the study in the *Alpare*

Parietaria group (17 of 19, 89%) than in the placebo group (2 of 17, 12%). One patient in the placebo group experienced a reaction which was classified as a systemic reaction. No treatment was necessary for this reaction and the symptoms described were vague. No objective symptoms of anaphylaxis were reported. Of those patients who completed 2 years of the study, all nine (100%) active treatment patients experienced local reactions. One of the placebo patients experienced a systemic reaction.

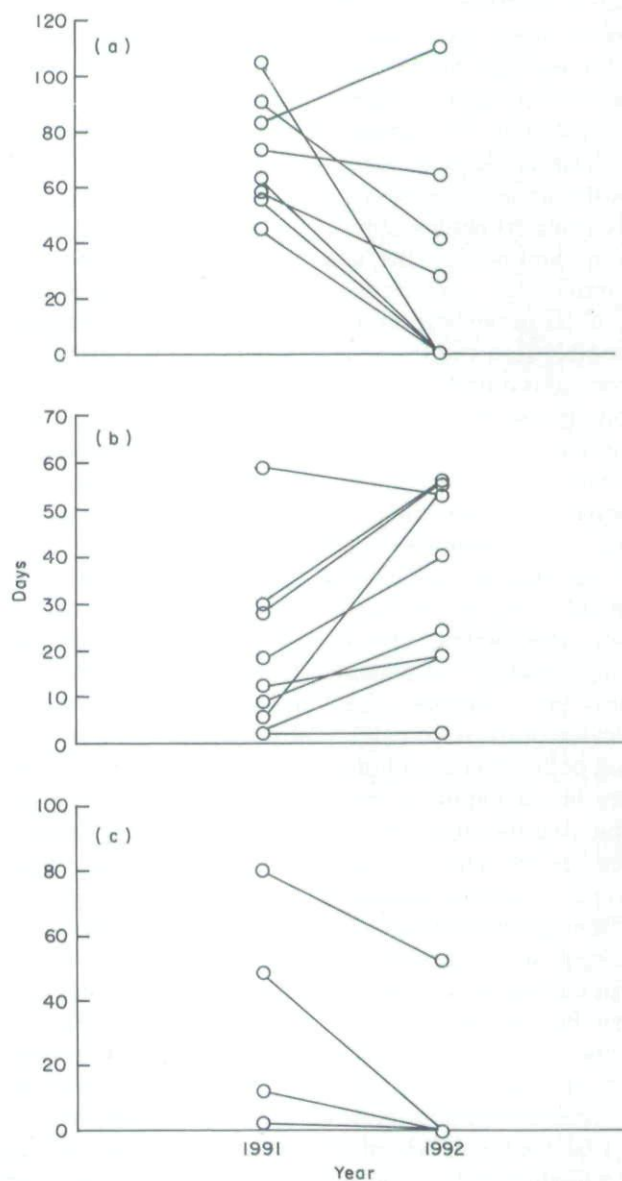


Fig. 5. Medication use for 1991 and 1992 for placebo patients. Results indicate days of medication use during the pollen seasons for 1991 and 1992. (a) Nasal spray. (b) Antihistamines. (c) Other medications.

End of study status

Significantly ($P < 0.01$) more patients in the Alpare *Parietaria* group assessed their rhinitis symptoms as being better or much better (eight of nine, 89%) compared with the placebo group (0 of 11, 0%).

Discussion

Parietaria pollen allergens are a prevalent cause of allergy in the Mediterranean area and are the most important pollen allergen in some European regions such as southern Italy (in particular the Naples area), coastal Spain and Greece [15,17]. *Parietaria* species also occur outside the Mediterranean area and have been identified in other European countries, in the USA and in Australia [36–39]. Among the various *Parietaria* species, *Parietaria judaica* is the most common in Mediterranean coastal areas. *Parietaria* pollen, which has very strong allergenic properties, appears first at the beginning of the spring and persists throughout the spring and summer months often reaching peak levels of about 500 grains/m³ of air in the beginning of June. A shorter pollination period is observed from the end of August to October. A prodigious output of pollen, which ensures the reproduction success of the plant, is responsible for frequently severe respiratory symptoms and asthma. In Naples, the prevalence of sensitivity to *Parietaria* as an isolated allergen or in association with other allergens, is about 80% of all pollinosis patients [17].

The incidence of asthma in addition to rhinitis in patients who are allergic to *Parietaria judaica* is quite high, approaching 60% in some areas [17,19]. In our study, seven of 20 patients (35%) who completed the study had a history of asthma in addition to rhinitis. Because of the large amount of pollen produced and the long pollinating season in some areas, *Parietaria* allergy may be an important risk factor for asthma. For the same reasons, successful immunotherapy for the treatment of *Parietaria* induced allergic symptoms would have a significant clinical impact.

Immunotherapy studies providing information on the strength of the extract used and the adequacy of the maintenance dose [1–4] show that high doses are necessary for successful immunotherapy. In our study we employed an extract standardized by skin testing sensitive patients and comparing the reactions to those obtained from a histamine control. The extract strength was labelled in AURs/ml [31]. Because the standardization method is the same as is used to determine BUs we are able to express the strength of the extract in BUs. BUs have been used as a standardization unit in a number of immunotherapy studies. The targeted maintenance dose in our patients was 12 500 BUs in the

actively treated group. Expressing the strength in this manner allows one to compare the results of this study with prior or subsequent ones that might be performed using extracts standardized in BUs.

The data suggest this dose is efficacious. The skin test part of the study showed a decrease in skin reactivity in the actively treated vs the placebo group. The suppression was greatest at higher concentrations where there was more room for change and more suppression was seen after year 2 than after year 1. We chose to monitor skin changes by measuring patient reactivity to specific doses rather than using a parallel line bioassay technique because it was easier in the clinic to measure skin reactivity from pre-selected doses. For the parallel line bioassay methodology preliminary skin testing must be performed to determine each patient's sensitivity so specific skin testing can be performed on the steep part of the dose response curve. By pre-selecting specific strengths the necessity for the preliminary skin testing was eliminated.

The VAS scores are an easy way to determine patient response to immunotherapy. Patients are simply asked to rate their symptoms at any given time by visually estimating symptom severity on a line which is 10 cm in length. It is easy to get patient compliance as little effort on the part of the patient is required. Our study showed a definite decrease in the VAS measured symptomatology in the actively treated vs the placebo group.

Nasal provocation tests are useful in judging the adequacy of immunotherapy. These challenges provide objective data which may be important clinically. Currently, there is no accepted standardized method for nasal provocation tests. The American Academy of Allergy Committee on Upper Airway Allergy suggested that results of nasal provocation tests should be interpreted with caution [40]. Rhinomanometry alone is not reliable to measure the results of nasal provocation tests because patients exhibit much variability in mucous production and obstruction after nasal challenges. The nasal provocation procedure employed in this study did not use rhinomanometry measurements but relied on patient symptoms and physical examination. A significant improvement was seen in the actively treated group vs the placebo group. This information is consistent with the other efficacy observations which were made throughout the study.

Useful patient diaries are difficult to obtain if the study is performed over a long period of time, as this study was since diaries require the patient to record information over a specified time period. A review of the diaries revealed that multiple data points appeared to have been recorded at one time in several instances. The patient must be extremely motivated to record the

symptom scores for each day at the end of the day. Even with the difficulties inherent with collecting data in this way, patients in the actively treated group showed a significant decrease in symptomatology compared with placebo. The scores were higher for the placebo patients throughout both years of the study. During the peak of the pollen season in 1991 the placebo group showed an increase in symptomatology as would be expected. By 1992 the scores in the placebo group were still greater than those in the actively treated group. The 1992 pollen season showed a different pattern than in 1991 with peak levels occurring in early May and a second lesser peak occurring in June.

Reactions to the therapy employed in this study were not severe and consisted of local reactions only. The incidence of such reactions was significantly greater in the actively treated group vs the placebo group. One would expect to see such local reactions if a strong extract were used for immunotherapy. These reactions suggest that the strength of the extract used for this study was adequate to produce local reactions but not so strong as to produce systemic reactions.

The end of study status is another measurement which is easy to perform. Patients are simply asked if they feel better than they did prior to beginning immunotherapy. It is very easy to get patient compliance because if the patient has continued to receive immunotherapy to that particular time point he or she is simply asked if their symptoms are much better, better, unchanged, worse or much worse than prior to beginning immunotherapy. Significantly more patients in the actively treated group were much better or better as compared with the placebo group.

Overall the study suggests that treatment of *Parietaria* rhinitis with an allergenic extract used at a maximum targeted maintenance dose of 12 500 BUs (80 AURs) is efficacious and safe. Because such a high percentage of patients with *Parietaria* induced rhinitis also have asthma, patients who have asthma due to *Parietaria* allergy would constitute a good group to access the effect of immunotherapy on asthma.

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References

- 1 EAACI Subcommittee, Steering Committee. Immunotherapy Position paper Allergy 1993; 48(Suppl):9-36.

- 2 BSACI Working Party: Allergen immunotherapy (Position paper) Clin Exp Allergy 1993; 23(Suppl. 3):1-44.
- 3 Bousquet J, Michel FB. Advances in specific immunotherapy (Review). Clin Exp Allergy 1992; 22:889-96.
- 4 Norman PS. Immunotherapy. Progr Allergy 1981; 32:318-55.
- 5 Kay AB. Allergen injection immunotherapy (hyposensitization) on trial. Clin Exp Allergy 1989; 19:591-6.
- 6 Norman PS. Immunotherapy for nasal allergy. J Allergy Clin Immunol 1988; 81:992-6.
- 7 Ewan PW. Allergen Immunotherapy. Curr Opin Immunol 1989; 1:672-8.
- 8 Norman PS. Immunotherapy. In Melillo G, Norman PS, Marone G (eds). Respiratory Allergy. Toronto, BC: Decker, 1990:3-15.
- 9 Bousquet J, Michel FB. Specific immunotherapy — a treatment of the past? ACI News 1980; 1:7-10.
- 10 Grant IWB. Does immunotherapy have a role in the treatment of asthma? Clin Allergy 1986; 16:7-10.
- 11 Mosbech H, Weeke B. Does immunotherapy have a role in the treatment of asthma? Clin Allergy 1986; 16:11-6.
- 12 Thompson R, Bousquet J, Cohen S et al. The current status of allergen immunotherapy (hyposensitization). Report of WHO-IUS Working Group. Lancet 1989; 1:259-61.
- 13 Committee on Safety of Medicine. Sensitizing vaccines. Br Med J 1986; 293:948.
- 14 Norman PS. An overview of immunotherapy, implications for the future. J Allergy Clin Immunol 1980; 65:87-96.
- 15 D'Amato G, Ruffilli A, Sacerdoti G, Bonini S. Parietaria pollinosis — a review. Allergy 1992; 47:443-9.
- 16 D'Amato G, Liccardi G, Melillo G. A study of airborne allergenic pollen content of the atmosphere of Naples. Clin Allergy 1983; 13:537-44.
- 17 D'Amato G, Lobefalo G. Allergenic pollens in the Mediterranean area. J Allergy Clin Immunol 1989; 83: 116-22.
- 18 D'Amato G, Dal Bo S, Bonini S. Pollen related allergy in Italy. Ann Allergy 1992; 68:433-7.
- 19 D'Amato G, Ruffilli A, Ortolani C. Allergenic significance of Parietaria (Pellitory-of-the-wall) pollen. In: D'Amato G, Spieksma F Th M, Bonini S (eds). Oxford: Blackwell Scientific Publications 1991:111-6.
- 20 Varney VA, Gaga M, Frew AJ et al. Usefulness of immunotherapy in patients with severe summer hay fever uncontrolled by anti allergic drugs. Br Med J 1991; 302: 265-9.
- 21 Bousquet J, Hejjaoui A, Soussana M, Michel FB. Double blind placebo controlled immunotherapy with mixed grass pollen allergoids. IV. Comparison of the safety of two dosages of a high molecular weight allergoid. J Allergy Clin Immunol 1990; 85:490-7.
- 22 Brunet CV, Bedard PM, Lavoie A et al. Allergic rhinitis to ragweed pollen. Reassessment of the effects of immunotherapy on cellular and humoral responses. J Allergy Clin Immunol 1992; 89:76-86.
- 23 Juniper EF, Kline PA, Ramsdale EH, Hargreave FE. Comparison of the efficacy and side effects of aqueous

- steroid nasal spray (budesonide) and allergen-injection therapy (Pollinex-R) in the treatment of seasonal allergic rhino-conjunctivitis. *J Allergy Clin Immunol* 1990; 85:606–11.
- 24 Ortolani C, Pastorello EA, Incorvaia C et al. A double-blind placebo controlled study of immunotherapy with an alginate-conjugated extract of *Parietaria judaica* in patients with *Parietaria* hay fever. *Allergy* 1994; 49:13–24.
- 25 Andri L, Senna GE, Betteli C et al. Local nasal immunotherapy in allergic rhinitis to *Parietaria*. A double-blind controlled study. *Allergy* 1992; 47:318–23.
- 26 Creticos PS, Adkinson NF Jr, Kagey-Sobotka A et al. Nasal challenge with ragweed pollen in hay fever patients: effect of immunotherapy. *J Clin Invest* 1985; 76:2247–54.
- 27 Van Bever HP, Stevens WJ. Evolution of the late asthmatic reaction during immunotherapy and after stopping immunotherapy. *J Allergy Clin Immunol* 1990; 86:141–6.
- 28 Ilipoulos O, Proud D, Adkinson NF et al. Effects of immunotherapy on the early, late and rechallenge nasal to provocation with allergen — changes in inflammatory mediators and cells. *J Allergy Clin Immunol* 1991; 87: 855–66.
- 29 Creticos PS, Marsh DG, Proud D et al. Responses to ragweed-pollen nasal challenge before and after immunotherapy. *J Allergy Clin Immunol* 1989; 84:197–205.
- 30 Majchel AM, Proud D, Freidhoff L et al. The nasal response to histamine challenge — effect of the pollen season and immunotherapy. *J Allergy Clin Immunol* 1992; 90:85–91.
- 31 Watson HK, Rooke MB. A proposed unitage system for allergen extracts. *Allergy* 1982; 37(suppl. 1):24.
- 32 Nordic Guidelines. By the Nordic Council on Medicines as a basis for national regulations. 2nd Edition Nov. 1989 (HLN — Publication No. 7).
- 33 Cochran WG, Cox GM. *Experimental Designs*, New York: John Wiley & Sons, Inc. 1950.
- 34 Fisher RA. *Statistical Methods for Research Worker*, 5th Edition, Edinburgh: Oliver & Boyd. 1934.
- 35 Satterthwaite FW. 'An Approximate Distribution of Estimates of Variance Components'. *Biometrics Bulletin* 1946; 2:100–14.
- 36 Holgate SJ, Jackson L, Watson HK, Ganderton M. Sensitivity to *Parietaria* pollen in Southampton area as determined by skin prick and RAST tests. *Clin Allergy* 1988; 18:549–56.
- 37 Ford SA, Baldo BA, Geraci G, Bass D. Identification of *P. judaica* pollen allergens. *Int Arch Allergy Appl Immunol* 1986; 79:120–6.
- 38 Kaufman HS. *Parietaria*: an unrecognized cause of respiratory allergy in the United States. *Ann Allergy* 1990; 64:293–6.
- 39 Bass D, Baldo BA. *Parietaria* as a cause of asthma. *Med J Aust* 1984; 14:511–4.
- 40 Druce HM, Schumacher MJ. Nasal Provocation Challenge. Report of the committee on upper airway allergy. *J Allergy Clin Immunol* 1990; 86:261–4.

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