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A randomized, controlled study of specific immunotherapy in monosensitized subjects with seasonal rhinitis: effect on bronchial hyperresponsiveness, sputum inflammatory markers and development of asthma symptoms

Nunzio Crimi, Fabrizio Li Gotti, Giuseppe Mangano, Giuseppina Paolino, Claudio Mastruzzo, Carlo Vancheri, Natalina Lisitano, Riccardo Polosa

Allergic rhinitis is often associated with bronchial hyperresponsiveness (BHR) and airway inflammation, and it seems to be an important risk factor for the development of asthma. Specific immunotherapy (SIT) reduces symptoms and medication requirements in subjects with allergic rhinitis, but the mechanisms by which SIT promotes these beneficial effects are less clear.

We have investigated the effects of *Parietaria*-SIT on rhinitis symptoms, BHR to inhaled methacholine, eosinophilic inflammation and cytokine production (interferon γ and interleukin-4) in the sputum. The effect on asthma progression was also examined.

Thirty non-asthmatic subjects with seasonal rhinitis and monosensitized to *Parietaria judaica* participated in a randomized, double-blind, placebo-controlled, parallel group study. Participants were randomly assigned to receive injections of a *Parietaria* pollen vaccine (n = 15) or matched placebo injections (n = 15) in a rapid up dosing cluster regimen for 7 weeks, followed by monthly injections for 34 months. Throughout the 3-year study we collected data on symptoms and medication score, airway responsiveness to methacholine, eosinophilia and soluble cytokines in sputum, followed by a complete evaluation of the clinical course of atopy.

Hay fever symptom and medication scores were well controlled by SIT. By the end of the study, in the placebo group, symptom and medication scores significantly increased by a median (interquartile range) of 121% (15-280%) and 263% (0-4400%) respectively ($p < 0.01$), whereas no significant difference was observed in the SIT group. We found no significant changes in the sputum parameters and methacholine PC₁₅ values in both groups throughout the study. By the end of the investigation, a total of 9 out of 29 participants developed asthma symptoms; of these, seven (47%) belonged to the placebo group, whereas only 2 (14%) to the SIT-treated group ($p = 0.056$).

In conclusion, *Parietaria*-SIT is effective in controlling hay fever symptoms and rescue medications, but no changes in the BHR to methacholine or sputum eosinophilia were observed. Moreover, *Parietaria*-SIT appears to prevent the natural progression of allergic rhinitis to asthma, suggesting that SIT should be considered earlier in the management of this condition.

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Key words: Bronchial hyperresponsiveness; Induced sputum; *Parietaria* allergy; Seasonal allergic rhinitis; Specific immunotherapy; T_{H1}/T_{H2} balance.

Introduction

Allergic rhinitis is one of the most common chronic conditions in the industrialized world and is often associated with several comorbidities that include allergic conjunctivitis, sinus disease, otitis media, and asthma¹⁻³. In a proportion of allergic individuals with rhinitis, a bronchial challenge with histamine or methacholine may reveal bronchial hyperresponsiveness (BHR) even in the absence of any asthmatic symptoms⁴⁻⁶ and this may be a reflection of subclinical inflammatory changes in the lower air-

ways⁷⁻¹⁰. There is evidence that BHR may help to identify subjects with rhinitis who are at risk for asthma progression¹¹⁻¹³. In addition, a number of epidemiological surveys suggest that allergic rhinitis may prelude to airway symptoms related to asthma^{3,14,15}.

Allergen-specific immunotherapy (SIT) is a widely used form of therapy for allergic conditions¹⁶. Although the evidence of its effectiveness in asthma remains controversial¹⁷, its efficacy in reducing the severity of symptoms related to allergic rhinoconjunctivitis is well established^{16,18}. Grass pollen immunotherapy improves symptoms and reduces medication requirements in subjects with severe seasonal allergic rhinitis and results in long-

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term clinical benefit for at least 3 years after discontinuation of treatment^{18,19}. It is also possible that when given prophylactically to susceptible individuals, SIT may be effective in preventing progression to asthma rather than reversing its course once the disease is established^{20,21}. The reasons for these beneficial effects are not known, but one explanation rests on the potential of SIT to attenuate BHR and airway inflammation.

The association between T_{H2}-type cytokine production and an allergic phenotype on the one hand, and T_{H1}-type cytokine production with non-atopic “protective” responses on the other is well established²²⁻²⁴. It is therefore plausible that SIT may modify the T-cell response to subsequent natural allergen exposure by shifting the balance of T-cell subsets away from T_{H2}-type (producing particularly interleukin-IL-4 and IL-5) in favor of a T_{H1}-type response (with preferential production of interferon-IFN γ)²⁵. Such a deviation in the T-cell effector phenotype may also be expected to account for the inhibition of cytokine-mediated eosinophil recruitment, activation, and persistence in tissues.

We have conducted a randomized, placebo-controlled 3-year study of SIT in non-asthmatic subjects with rhinitis monosensitized to *Parietaria* pollen with documented seasonal increases in non-specific BHR and sputum eosinophils during the pollen season. Subjects allergic to *Parietaria* were selected, as it appears to be the major cause of respiratory allergy in the Mediterranean area and particularly in Sicily where the study was carried out²⁶.

Outcome measures included seasonal symptoms, use of rescue medication, bronchial responsiveness to metha-

choline, eosinophilic airway inflammation in sputum, and T_{H1} (IFN γ) and T_{H2} (IL-4) cytokine levels in the fluid phase of sputum. The possible progression of allergic rhinitis to asthma in both treatment groups was also assessed.

Methods

Study design

The present study was a parallel group, double-blind, placebo-controlled trial lasting for a period of 3 years (December 1997-December 2000) during which we investigated the effects of SIT with high-dose standardized *Parietaria judaica* extract on: 1) symptom and medication score; 2) airway methacholine responsiveness; 3) eosinophilia and soluble cytokines in sputum, and 4) the clinical course of atopy, in a group of patients with rhinitis who were allergic to *Parietaria* (Fig. 1). The local Ethics Committee approved the study, and written informed consent was obtained from each subject.

Upon enrolment (December 1997, out of *Parietaria* season), a detailed history of each subject was taken and physical examination, spirometry, and bronchial challenges with methacholine were carried out. Sputum induction was performed at least 1 week later. Subjects were randomized to receive either active treatment, consisting of increasing doses of allergen extract given subcutaneously, followed by monthly maintenance treatment, or placebo. The randomization sequence was generated by the supplier of the *Parietaria* pollen vaccine (ALK-Abellò, Milan,

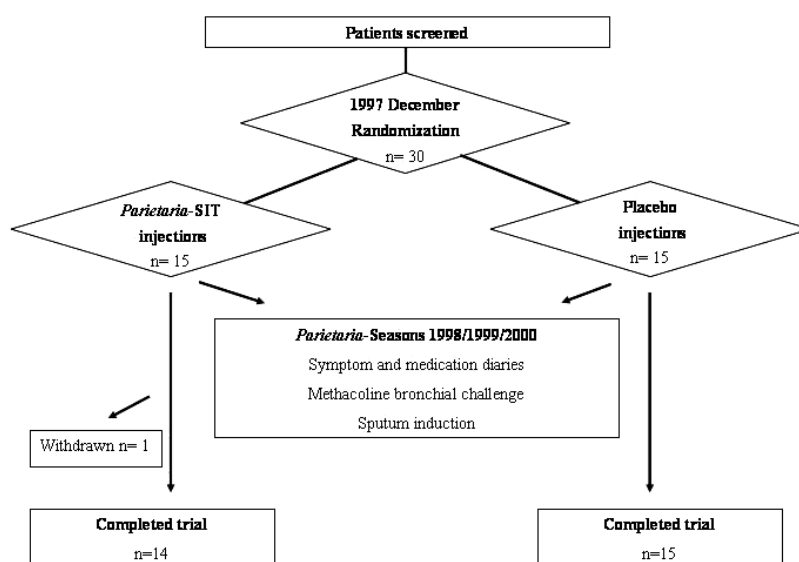


FIGURE 1. Patient flow-chart illustrating the timing of assessments. One patient in the specific immunotherapy (SIT) group was lost to follow-up due to a change in residence.

Italy) using a random number generator. The treatment schedule and assessments were performed in a double-blind manner, with treatment allocations kept in sealed envelopes by the principal investigator. From March to July 1998, 1999, and 2000 the participants recorded their daily symptoms and rescue medications on diary cards. Bronchial challenges with methacholine and sputum induction were repeated during the peak of the *Parietaria* season in 1998, 1999 and 2000. Relief medications for symptoms of rhinitis were withheld for at least 3 days before each study visit.

Patients

Thirty non-smoking subjects (15 females and 15 males, mean age 33 years, age range 20-54 years) with a documented history of seasonal allergic rhinitis were recruited from the Allergy Clinic of our Institution (Table I). These subjects were selected on the basis of positive skin prick tests (wheal > 3 mm) to *Parietaria judaica* and negative tests to a panel of 21 common aeroallergens (including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, grass, trees, dog fur, cat fur, *Olea europea*, *Aspergillus fumigatus*). Exclusion criteria were a past or present history of asthma, previous asthma symptoms or asthma medication intake, and/or abnormal spirometric values. Since excluding the diagnosis of asthma was an important component of our study, we opted for extremely rigorous exclusion criteria for asthma, which was defined as at least 2 reported episodes of breathlessness, cough, and wheeze within the past 12 months. The possibility of unrecognized asthma in the participants of our study was categorically excluded by further reviewing their case histories and subjects were eligible for inclusion

in the study if at least two specialists in allergic diseases agreed that they did not have any clinical history or symptoms suggestive of asthma. None of the subjects studied had ever received SIT. During the course of the study, subjects seeking symptomatic relief were allowed only oxymetazoline spray and/or 10 mg loratadine tablets. Throughout the study, subjects were requested not to take nasal and oral corticosteroids.

Study outcome variables

Assessment of disease activity. A daily record of allergic symptoms and use of relief medications was kept throughout each *Parietaria* pollen season (March-July) for 3 consecutive years (1998-2000). Nasal/conjunctival symptoms were scored on a scale of 0 to 3 (0 = no symptoms; 1 = mild; 2 = moderate; 3 = severe) for 5 different items (sneeze, blockage, itch, running and eye streaming/itching) and totaled daily. Lower respiratory symptoms (breathlessness, cough, wheeze) were also recorded and scored on a scale of 0 to 1 (0 = absent; 1 = present). Asthma was defined as the recurrence of at least 2 of the following 3 symptoms: breathlessness, cough, and wheeze. Daily medication scores were totaled for the allowed rescue treatments: each actuation ($\times$ 2 nostrils) of oxymetazoline and each inhalation (2 puffs) of salbutamol was scored 1; each 10-mg loratadine tablet was scored 2. During the course of the study, the subjects were requested not to take nasal and oral corticosteroids. In order to maintain the blindness of the study protocol, the physicians who consulted the participants were not involved in the data analyses.

Measurement of airway responsiveness. The forced expiratory volume in 1 second (FEV₁), and forced vital capacity were measured with a computerized spirometer (Cosmed Altair Vega Hercules Delta III, Cosmed Srl, Pavona di Albano, Rome, Italy) and the results were expressed as percent of predicted.

BHR was evaluated by methacholine bronchial challenge, as described previously²⁷. In brief, methacholine (Lofarma, Milan, Italy) was dissolved in phosphate-buffered saline (pH 7.4) to produce increasing doubling concentrations (0.125-16 mg/mL) and immediately used for bronchial challenge. The solutions were administered as aerosols generated from a starting volume of 3 mL in a disposable Inspiron Minineb (C.R. Bard International, Sunderland, UK) driven by compressed air at 8 L/min. Subjects inhaled the aerosolized solutions in five breaths from the functional residual capacity to total lung capacity via a mouthpiece. Subjects were trained to reach the total lung capacity in 3 s. After five breaths of diluent solu-

TABLE I. Clinical characteristics of participants.

	<i>Parietaria</i> -SIT (n = 15)	Placebo (n = 15)
Sex (M/F)	10/5	5/10
Age (years)	32 (21-54)	34 (20-53)
Duration of rhinitis (years)	7.8 (4-14)	8.2 (4-16)
Seasonal nasal symptoms scores 1998*	140 (80-240)	133 (78-176)
Seasonal medication scores 1998*	12 (6-47)	18 (0-58)
Positive response to methacholine [†]	5/15	10/15
Eosinophils in sputum (%)	3.0 (0.5-6.5)	4.5 (1.5-5.0)

Median values are given for scores and percentage eosinophils; interquartile range in parentheses. Mean values are given for age; range in parentheses.

SIT = specific immunotherapy.

* scores derived at the peak of the *Parietaria* pollen season 1998 and expressed as the area under the curve; [†] PC₁₅ methacholine < 16 mg/mL.

tion, subjects inhaled increasing doubling concentrations of agonist. The FEV₁ was measured at 1 and 3 min after administration of each concentration of agonist and the better of the two values recorded for analysis. The challenges were stopped when a 15% decrease in the FEV₁ had been achieved or when the maximum concentration of agonist had been inhaled. The bronchial responses to the inhaled agonists were expressed as the provocative concentration causing a 15% decline in the FEV₁ (PC₁₅) which was calculated by linear interpolation from the concentration-response curve constructed on a logarithmic scale by plotting the percentage change in the FEV₁ from the post-diluent value against the cumulative concentration of agonist administered.

Sputum induction and processing. Induction was performed according to our previously published method^{10,28}. Briefly, participants inhaled hypertonic saline (4.5%) aerosolized by an ultrasonic nebulizer (UltraNeb 99, DeVilbiss, Feltham, Middlesex, UK) with the output set at 3 mL/min. The subjects wore a nose clip and quietly inhaled the aerosol for up to five consecutive 5-min periods until an adequate volume of sputum was collected.

The sputum plugs were transferred into 50 mL polypropylene tubes (Becton Dickinson, Abingdon, UK), weighed, and an equal weight of 0.01 M dithioerythritol (Fluka, Gillingham, Dorset, UK) solution added to dissolve the mucus. Specimens were then vortexed for 10 s, rocked for 30 min at room temperature, and again vortexed for another 10 s. They were then filtered through a 70-µm strainer (Becton Dickinson) and the collected fluid centrifuged at 400 g for 10 min at 4°C. The supernatants were removed and stored at -70°C. The cell pellets were resuspended in 1 mL of phosphate-buffered saline without Ca²⁺ and Mg²⁺ and viable cells counted in a hemocytometer. Only samples in which squamous cells comprised < 30% of the total cells were considered satisfactory for analysis. Differential counting was carried out using cytopins stained with May-Grunwald-Giemsa on 600 cells (excluding squamous cells). Slides were coded and examined by one investigator and counts expressed as a percentage of the total number of cells and as absolute counts. IL-4 and IFNγ levels were measured in duplicate using commercially available ELISA kits (Bender Med Systems, Vienna, Austria) with a sensitivity of 10 and 1.5 pg/mL respectively.

Specific immunotherapy

A standardized extract of *Parietaria judaica*, aluminum adsorbed for slow release (Alutard SQ, ALK-Abellø), was used as SIT. The placebo injections were identical and

contained 0.01 mg/mL of histamine acid phosphate in allergen diluent (phosphate-buffered saline). Both preparations were administered by physicians who were not involved in the acquisition or analysis of either the clinical or physiologic data of the study. The same physicians were also responsible for the observation and treatment of any adverse reactions.

A modified "cluster" regimen of injections was given between December 1997 and February 1998 (Table II), followed by monthly maintenance injections until December 2000 which were adjusted in accordance with the published guidelines. In most of the participants, a maintenance dose of 80 000 standard quality units was achieved. Each 0.8-mL maintenance injection of 80 000 standard quality units was equivalent to 8000 biologic units and contained 4.8 µg of the major allergen *Par J1*. Local cutaneous reactions at the injection site and any other symptoms occurring within 30 min were documented. Subjects recorded any delayed (within 48 hours) local or generalized symptoms.

Pollen counts

A Burkard 7-day volumetric spore trap (Burkard, Rickmansworth, UK) placed on the exposed roof of our Institute was used to collect pollen grains. *Parietaria* pollens were measured daily and expressed as grains per cubic meter of air. Weekly average counts were calculated for the whole duration of the study. In 1998 and 2000, weekly *Parietaria* pollen counts in Catania peaked in March and May in the average range. The pollen counts for *Parietaria* were comparable in 1998 and 2000, but the

TABLE II. Cluster specific immunotherapy up dosing schedule.

Vial no.	Injection no.	Day	Hour	Volume injected (mL)
I (100 USQ/mL)	1	1	8:30	0.2
	2	1	9:30	0.6
II (1000 USQ/mL)	3	8	8:30	0.2
	4	8	9:30	0.6
III (10 000 USQ/mL)	5	15	8:30	0.2
	6	15	9:30	0.4
	7	22	8:30	0.6
	8	22	9:30	0.8
IV (100 000 USQ/mL)	9	29	8:30	0.2
	10	36	8:30	0.4
	11	43	8:30	0.6
	12	50	8:30	0.8

USQ = standard quality units.

Injections were given at 1-hour intervals and patients were kept under observation for 40 min after the last injection of each cluster. Monthly maintenance injections were administered for a further 34 months with a 50% dose reduction during the *Parietaria* pollen season.

area under the curve (AUC) showed a slightly higher (11.8%) value in 2000.

Statistical analyses

Symptom and medication scores were summed up for the 8 weeks corresponding to the peak pollen counts each year and expressed as AUC. The AUC data and all the variables in the sputum, which were not normally distributed, were expressed as medians (interquartile ranges). PC₁₅ values were logarithmically transformed to normalize their distribution and expressed as geometric mean (range).

For all the non-parametric data (AUC values and sputum variables), differences between groups over the 1998-2000 *Parietaria* pollen seasons were compared using Kruskal-Wallis one-way analysis of variance followed by the Mann-Whitney U test, whereas within groups comparisons were analyzed using Friedman's test followed by Wilcoxon's matched-pairs signed rank test where appropriate. Logarithmically transformed data (PC₁₅ values) were analyzed using two-way analysis of variance (ANOVA) followed by the paired Student's t-test. Bonferroni's correction was applied to allow for multiple comparisons. The Spearman's correlation test was used to analyze the relationship between different variables. The Fisher's exact test was used to compare the frequency of development of asthma in the two study groups.

The relationships between asthma progression and PC₁₅ values or percent sputum eosinophils at the beginning of the study were analyzed using the independent samples t-test. Binary logistic regressions were performed to evaluate whether PC₁₅ values and percent sputum eosinophils could forecast the beneficial effects of SIT. The odds ratio calculated from the logistic regression was used to assess the probability of preventing asthma progression in the SIT and placebo groups.

A two-tailed p value of < 0.05 was considered as statistically significant. All analyses were performed using the Statistical Package for Social Science (SPSS, Chicago, IL, USA) for Windows version 10.0.

Results

Patients

Twenty-nine of the 30 participants completed the 3-year study period (Fig. 1). One patient who was receiving SIT was lost to follow-up in the last year of the study because of a change in residence. The clinical characteristics of the participants are summarized in Table I. The patient groups were comparable with respect to age, airway responsiveness to methacholine, severity of symptoms and sputum eosinophilia.

Safety of *Parietaria* specific immunotherapy

The treatment was well tolerated, and no systemic reactions occurred throughout the study. No immediate or late systemic reactions were observed during either the induction or maintenance phase of treatment. Twelve delayed mild local reactions (wheal > 5 cm) were documented during the induction period, 9 in the actively treated group and 3 in the placebo-treated group. During maintenance treatment there were 3 delayed large local reactions (wheal > 10 cm) in the SIT-treated group and none in the placebo group. The reactions required no treatment, and all subjects continued in the study without further incidents.

Effects of specific immunotherapy on symptom and medication scores and on asthma progression

By the third year of the study, a significant difference between the symptom scores of the groups was observed; the median (interquartile range) symptom score of 310 (198-387) in the placebo group was 145 (55-210) in the SIT group ($p = 0.001$) (Fig. 2). Comparison of the 1998 data with those of the year 2000, showed a significant change in overall median symptom scores by 121% (15-280%) in the placebo group and by -16% (-64 to 37%) in the SIT group ($p = 0.001$).

Similarly, a significant difference in medication scores was observed between the two groups, the greatest effect being observed by the third year of the study. The median (interquartile ranges) medication scores were significantly lower in the SIT group than in the placebo group; 4 (0-110) vs 58 (16-116) in 1999 ($p = 0.041$; SIT vs placebo) and 5 (0-49) vs 78 (45-120) in 2000 ($p = 0.002$; SIT vs placebo) (Fig. 3). From 1998 to 2000, medication requirements decreased by 59% (-90 to 340%) in the SIT group and increased by 263% (0-4400%) in the placebo group ($p = 0.001$).

The time-dependent progression of asthmatic symptoms and of anti-asthma medication scores was apparent in subjects with seasonal allergic rhinitis. By the end of the investigation, a total of 9 out of 29 participants developed symptoms compatible with the diagnosis of asthma; of these, 7 (47%) belonged to the placebo group, whereas only 2 (14%) to the SIT group ($p = 0.056$).

Effects of specific immunotherapy on methacholine airway responsiveness

Between-group comparisons throughout the study showed no significant difference in PC₁₅ methacholine, their geometric mean (range) values being 5.21 (1.03-16) vs 6.36 (0.26-16) mg/mL ($p = 0.705$; placebo vs SIT, 1998), 3.86 (0.23-16) vs 8.01 (1.02-16) mg/mL ($p = 0.759$;

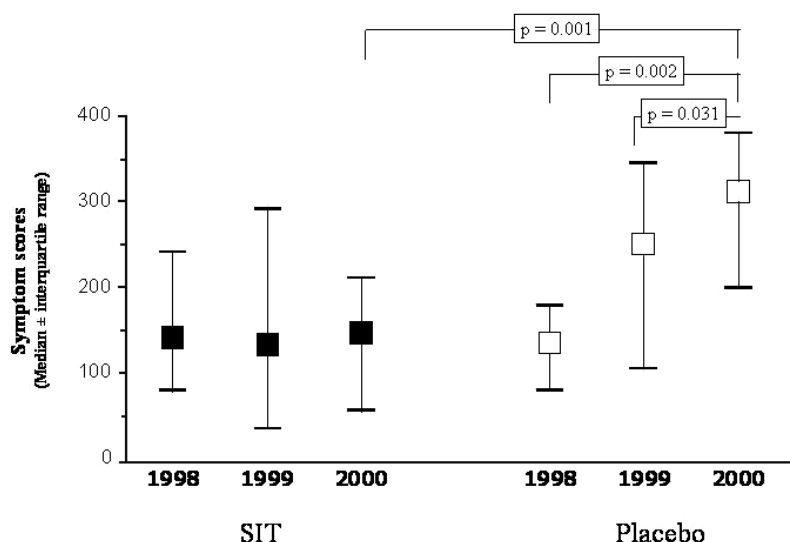


FIGURE 2. Changes in the median ($\pm$ interquartile range) symptom scores in the specific immunotherapy (SIT) (closed squares) and placebo-treated (open squares) groups at the peak of the Parietaria season in 1998, 1999, and 2000. A significant difference ($p = 0.001$) was observed by the third year of treatment.

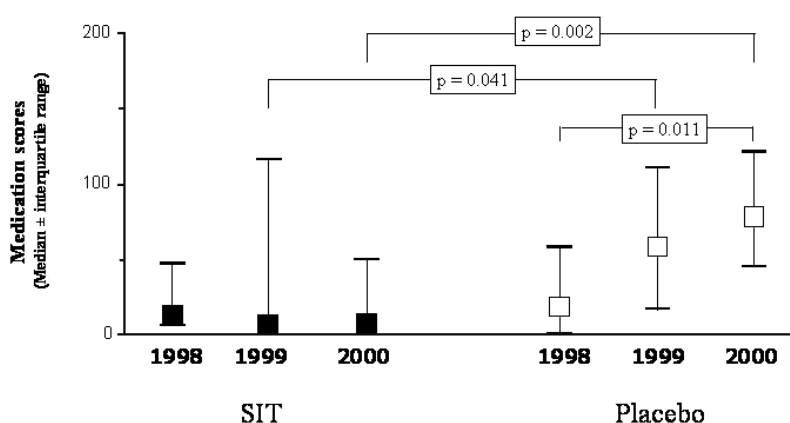


FIGURE 3. Changes in the median ($\pm$ interquartile range) medication scores in the specific immunotherapy (SIT) (closed squares) and placebo-treated (open squares) groups at the peak of the Parietaria season in 1998, 1999, and 2000. A significant difference was observed in the second ($p = 0.041$) and third years ($p = 0.002$) of treatment.

placebo vs SIT, 1999), and 5.64 (1.12-16) vs 6.89 (0.16-16) mg/mL ($p = 0.604$; placebo vs SIT, 2000) (Fig. 4). Similarly, no significant changes in PC₁₅ methacholine values were observed within the placebo and the SIT group.

Effects of specific immunotherapy on sputum eosinophils and on interferon γ /interleukin-4 concentrations

We obtained sputum samples on all but six occasions (three on 1999 and three on 2000). Complete data on the IFN γ and IL-4 concentrations in the sputum were available for 22 subjects (11 in the SIT group and 11 in the placebo group).

Between-group comparisons throughout the study showed no significant difference in percentage eosinophil counts, their median (interquartile range) values being 10.5% (3-30%) vs 12.0% (3.5-26%) ($p = 0.917$; placebo vs SIT, 1998), 10.0% (3.5-30%) vs 10.8% (1-63%) ($p = 0.819$; placebo vs SIT, 1999), and 17.0% (4.9-30.1%) vs 7.8% (1.8-46.5%) ($p = 0.402$; placebo vs SIT, 2000) (Fig. 5).

Further analyses revealed that sputum eosinophil ($p = 0.014$) but not methacholine PC₁₅ ($p = 0.108$) values at the beginning of the study are good predictors of subsequent asthma progression.

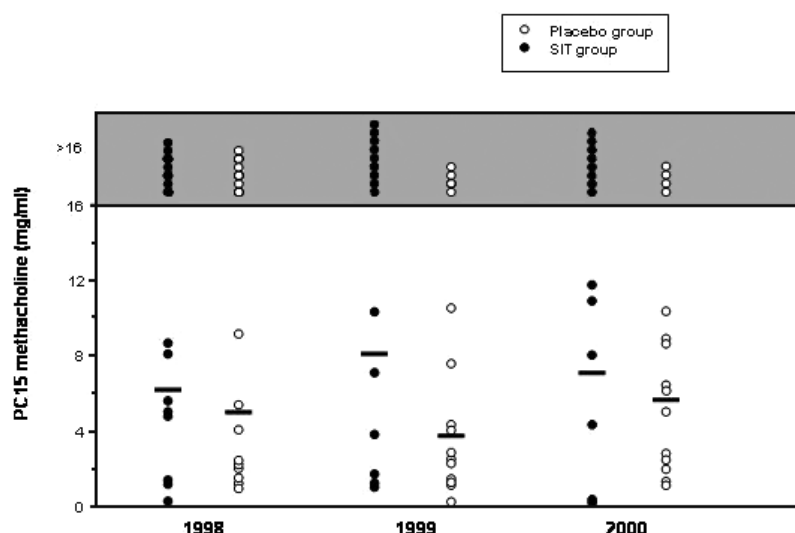


FIGURE 4. Individual methacholine PC₁₅ values in the specific immunotherapy (SIT) (closed circles) and placebo-treated (open circles) groups at the peak of the Parietaria season in 1998, 1999, and 2000. No significant between-group difference was observed in any phase of the study. The geometric mean values are shown as horizontal bars. Negative methacholine responders are represented in the shaded area.

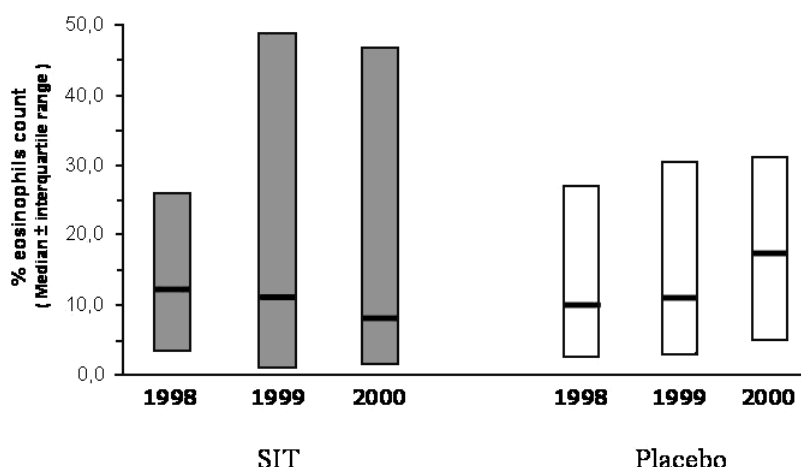


FIGURE 5. Changes in the median ($\pm$ interquartile range) percentage eosinophil counts in the specific immunotherapy (SIT) (gray bars) and placebo-treated (white bars) groups at the peak of the Parietaria season in 1998, 1999, and 2000. No significant between-group difference was observed in any phase of the study. Medians are shown as horizontal bars.

Sputum IFN γ and IL-4 concentrations between groups did not change significantly throughout the study period. By the end of the study, the mean ($\pm$ SEM) concentrations of sputum IFN γ and IL-4 were 7.6 ± 4.7 and 140.3 ± 31.9 pg/mL in the placebo group and 13.7 ± 5.3 and 137.6 ± 26.3 pg/mL in SIT subjects.

We did not find any correlation between sputum eosinophils and methacholine PC₁₅ ($r = -0.29$, $p = 0.116$). We found no significant correlation between IFN or IL-4

concentrations in the sputum and neither the percentage eosinophils count nor the PC₁₅ values at any time point of the study.

Discussion

The primary objective of this study was not to evaluate the clinical efficacy of SIT in allergic rhinitis, since this issue had already been addressed in other similar trials, but

to monitor the SIT-induced changes of the well-known surrogates of airway inflammation and of T_{H1}/T_{H2} cell balance. This was accomplished by prospectively following airways responsiveness to inhaled methacholine, sputum eosinophilia, and IL-4 and IFN γ production in the sputum of subjects receiving SIT with *Parietaria* allergen extracts. The restriction of the inclusion criteria to subjects who were sensitized to only one allergen (*Parietaria judaica*) is critical to the whole investigation because it provides a clean immunological model where to test our hypotheses. Moreover, we sought evidence for the prevention of asthma progression, and for this reason we selected only non-asthmatic subjects with rhinitis.

To improve acceptability for both subjects and medical staff, we adopted a rapid up dosing cluster injection protocol. This regimen was well tolerated, with all subjects reaching the intended maintenance dose within the planned 7 weeks. Although a number of mild and a few severe local reactions occurred, no systemic symptoms occurred during any phase of the study. We conclude that the modified cluster protocol was associated with minimal side effects and good compliance.

The results of the present study demonstrate that SIT is effective in controlling symptom and medication scores in allergic rhinitis subjects sensitized to *Parietaria* pollen. In the present study, the differences in symptom and medication scores between SIT and placebo-treated subjects were greater in 2000 than in 1999. This is likely to be a reflection of the longer duration of SIT which may have contributed to the larger differences observed between the groups after 3 years of treatment. The improvement in clinical symptom and medication scores was not associated with a decline in bronchial responsiveness to methacholine or with modifications in eosinophilic airway inflammation and IFN γ and IL-4 concentrations in the sputum. However, our data indicate that in subjects with seasonal allergic rhinitis who do not have any symptoms of asthma, SIT may prevent progression to asthma.

The evidence of the present study that SIT is beneficial in preventing the severity of the airway symptoms related to seasonal allergy due to *Parietaria* pollen is not novel as it has been already shown in previous randomized controlled trials²⁹⁻³¹. Perhaps the most interesting finding in the present study is that non-asthmatic subjects with seasonal allergic rhinitis progress to bronchial asthma and that SIT is likely to prevent the natural course of the disease. Although this effect of SIT was not significant ($p = 0.056$), a trend was clearly apparent with 47% of patients in the placebo group developing asthma symptoms by the end of the study, as opposed to only 14% of those in the SIT group. These findings support the evidence

that the use of immunotherapy in non-asthmatic individuals with rhinitis may reduce the occurrence of asthma^{21,32,33}. Moreover, in a recent retrospective survey of 371 consecutive non-asthmatic subjects with allergic rhinitis, we have reported that patients receiving immunotherapy have a 40% lower chance of developing asthma compared to untreated patients³⁴. It is unclear why a large proportion of individuals with atopy and rhinitis eventually progress to bronchial asthma. Although atopy *per se* carries an increased risk for the subsequent development of asthma in individuals with rhinitis, it is likely that chronic exposure to airborne allergens is important. The *Parietaria* pollen is widespread in the Mediterranean area with a very high frequency of sensitization (up to 80% in Sicily) and its long persistence in the atmosphere (in Sicily the *Parietaria* pollen season lasts from February to October) is often responsible for the almost perennial symptoms²⁶. As in previous studies with non-asthmatic subjects with allergic rhinitis^{9,10}, we have observed a substantial number of sputum eosinophils and an elevated BHR during periods of seasonal exposure to *Parietaria* pollen, which is known to reflect active allergic inflammation of the airways³⁵. Therefore ongoing exposure to *Parietaria* pollen may give rise to inflammatory changes in the bronchial airways of subjects with allergic rhinitis that may progress to asthma.

The reasons for the progressive worsening over the years of the study are not known, but may be related to the specific characteristics of the inhalant allergen type. In contrast to mite allergens, *Parietaria* pollen has very strong allergenic properties and reaches very high peak levels during season. Moreover, in a recent retrospective survey of 371 consecutive non-asthmatic subjects with allergic rhinitis³⁴, multivariate analysis demonstrated that sensitization to *Parietaria* is a significant independent risk factor for the worsening and progression of upper and lower airways symptoms.

The observed lack of effect of *Parietaria*-SIT against methacholine responsiveness of the airways confirms earlier findings by D'Amato et al.³⁶, who also failed to detect a significant effect of *Parietaria*-SIT with regard to non-specific BHR in subjects with seasonal asthma and rhinitis. However, our results are somewhat at variance with previous studies on mite and grass pollen allergy^{33,37,38}. The reasons for this discrepant effect of SIT on BHR are not quite clear, but are probably related to the characteristics of the inhalant allergen type. In contrast to mite allergens, *Parietaria* pollen, which has very strong allergenic properties, often reaches very high peak levels during season²⁶. Therefore, *Parietaria*-sensitive subjects are likely to be exposed to very high allergen levels, and this

high allergenic load may counterbalance the protective effect of SIT against the airways responsiveness to methacholine and sputum eosinophilia.

We also failed to detect a significant effect of *Parietaria*-SIT against sputum eosinophil counts. These findings are somewhat at variance with previous data on asthmatic subjects who are allergic to mite and birch pollen showing that immunotherapy may reduce the percentage of total and EG2+ eosinophils and the eosinophil cationic protein concentration in bronchoalveolar lavage and induced sputum^{39,40}. However, earlier findings by D'Amato et al.³⁶ demonstrate that there was no significant effect of *Parietaria*-SIT with regard to eosinophil cationic protein values in subjects with mild asthma and rhinitis. In addition, when sublingual immunotherapy to *Parietaria* pollen was administered to subjects with seasonal rhinoconjunctivitis, between-group comparisons failed to demonstrate a significant reduction of the eosinophilic infiltrate in the nasal brushings⁴¹. The rationale for the conflicting effects of SIT on sputum eosinophil counts remains unclear, but, as discussed before, is likely to be related to the peculiar characteristics of the *Parietaria* pollen season in the Mediterranean coastal areas.

One way of exploring the mechanisms of the beneficial effects of immunotherapy is to test the hypothesis that SIT elicits an immunologic shift in the balance of T-cell subsets away from the T_{H2}-type with a distinctive pattern in cytokine production. The effect of SIT against sputum IFN γ and IL-4 concentrations has never been addressed. In the present study, we failed to demonstrate that SIT converts the T_{H2} pattern of cytokine into a predominantly T_{H1} cytokine profile in treated subjects, since in no phase of the study did between-group comparisons show any significant difference. The shift from T_{H2} to T_{H1}, induced after immunotherapy treatment in atopic patients, has been proposed as one of the possible mechanisms through which SIT effectively operates²⁵. Work on stimulated allergen specific CD4+ T cells and peripheral blood mononuclear cells in cultures suggest that immunotherapy affects the T_{H1}/T_{H2} balance either by a decrease in T_{H2} cytokines (e.g. IL-4), or via an increase in T_{H1} cytokines (e.g. IFN γ)⁴²⁻⁴⁴. However, data regarding IFN γ production are conflicting⁴⁵. Others have reported on a more complex pattern whereby some patients have an increased and others have a decreased level of serum IL-4 after SIT⁴⁶, or that both T_{H1} and T_{H2} cytokines are suppressed⁴⁷. It is not clear why in the present study sputum IFN γ and IL-4 concentrations did not respond to *Parietaria*-SIT. Indeed, when compared to other inhalant allergens (e.g. house dust mite), a marked heterogeneity in terms of the functional T_{H1}/T_{H2} responses has been

described in patients allergic to *Parietaria*⁴⁸. Alternatively, the failure of SIT may be secondary to suppression of IL-12 production⁴⁹ or it may be due to a failure of immunotherapy to promote the expansion of regulatory T cells producing transforming growth factor- β ^{50,51}.

We conclude that *Parietaria* pollen injection immunotherapy followed by 3 years of maintenance treatment effectively controls hay fever symptoms and rescue medications. However, no changes in BHR to methacholine or sputum eosinophilia were observed. We have also shown that subjects with seasonal allergic rhinitis progress to bronchial asthma and that *Parietaria*-SIT is likely to slow down the natural course of the disease. Although larger studies are needed to confirm our observations and to define the characteristics of the patients who would benefit most from such a therapeutic approach, our findings indicate that SIT should be considered earlier in the treatment of rhinitis in order to prevent progression to more advanced and irreversible type of allergic diseases such as asthma.

Riassunto

La rinite allergica è spesso associata ad iperreattività bronchiale (BHR) e flogosi delle vie aeree, pertanto potrebbe rappresentare un importante fattore di rischio per lo sviluppo di asma bronchiale. L'immunoterapia specifica (SIT) migliora la sintomatologia e riduce l'uso di farmaci nei soggetti con rinite allergica anche se il meccanismo con cui ciò si verifica non è del tutto chiaro.

Abbiamo studiato gli effetti della SIT alla *Parietaria* nei confronti dei sintomi della rinite, BHR dopo stimolo con metacolina, flogosi eosinofila e rilascio di citochine (interferone γ e interleuchina-4) nello sputo indotto. Inoltre sono stati esaminati gli effetti sull'eventuale progressione verso asma bronchiale.

Trenta soggetti non asmatici, monosensibilizzati alla *Parietaria judaica*, affetti da rinite stagionale sono stati arruolati in uno studio randomizzato in doppio cieco e controllato con placebo. A questi soggetti veniva somministrata secondo un procedimento randomizzato una dose di vaccino con allergene della *Parietaria* (n = 15) o placebo (n = 15) con modalità di somministrazione rapida (completa in 7 settimane), seguita da somministrazioni mensili per i successivi 34 mesi. Nel corso dei 3 anni dello studio, abbiamo raccolto dati sui sintomi e l'impiego della terapia medica, BHR alla metacolina, presenza di eosinofili e citochine solubili nello sputo indotto e l'evoluzione clinica dell'atopia.

La SIT ha permesso un buon controllo della sintomatologia con una riduzione dell'impiego di farmaci. Al completamento dello studio, nel gruppo di controllo (pla-

cebo) gli score sintomatologici e di utilizzo dei farmaci mostravano un peggioramento con incremento significativo del valore di mediana (range interquartile) a 121% (15-280%) e a 263% (0-4400%) ($p < 0.01$) rispettivamente, mentre non si osservavano differenze significative nel gruppo trattato con SIT.

Inoltre non è stata messa in evidenza alcuna differenza nelle caratteristiche dell'espettorato e nei valori di PC_{15} dopo stimolo con metacolina in entrambi i gruppi per tutto il periodo dei 3 anni. A completamento dello studio, in 9 dei 29 partecipanti si è evidenziata la presenza di sintomi di asma bronchiale; di questi, 7 (47%) appartenevano al gruppo trattato con placebo e solamente 2 (14%) al gruppo trattato con SIT ($p = 0.056$).

La SIT alla *Parietaria* risulta efficace nel controllo dei sintomi della rinite e nella riduzione dell'impiego di farmaci. Tuttavia, non è stata osservata alcuna variazione della BHR dopo stimolo con metacolina né sono state evidenziate modifiche degli eosinofili nello sputo indotto. La SIT alla *Parietaria* risulta anche capace di prevenire la naturale progressione della rinite allergica verso l'asma bronchiale, suggerendone un impiego precoce nella gestione di tale patologia allergica.

Parole chiave: Allergia alla *Parietaria*; Bilanciamento T_H1/T_H2 ; Immunoterapia specifica; Iperreattività bronchiale; Rinite allergica stagionale; Sputo indotto.

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