

Short communication

Effect of immunotherapy on asthma progression, BHR and sputum eosinophils in allergic rhinitis

Background: Bronchial hyperresponsiveness (BHR) and airway inflammation are frequently associated with allergic rhinitis, and may be important risk factors for the development of asthma. Specific immunotherapy (SIT) reduces symptom in subjects with allergic rhinitis, but the mechanisms are not clear.

Aims of the study: To assess the effect of *Parietaria*-SIT on asthma progression, rhinitic symptoms, BHR, and eosinophilic inflammation.

Methods: Nonasthmatic subjects with seasonal rhinitis were randomly assigned to receive *Parietaria* pollen vaccine ($n = 15$) or matched placebo ($n = 15$).

Data on symptoms and medication score, BHR to methacholine, eosinophilia in sputum were collected throughout the 3-year study.

Results: By the end of the study, in the placebo group, symptoms 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 sputum eosinophils and BHR to methacholine in both groups throughout the study. Nine of 29 participants developed asthma symptoms during the study; of these, only two subjects (14%) in the SIT-treated group ($P = 0.056$).

Conclusions: *Parietaria*-SIT reduces symptom and rescue medication scores, but no changes in 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 subjects with allergic rhinitis.

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In a proportion of allergic rhinitic individuals, bronchial challenge with histamine or methacholine may reveal bronchial hyperresponsiveness (BHR) even in the absence of any asthmatic symptoms (1, 2) and this may be a reflection of subclinical inflammatory changes in the lower airways (3–5). There is evidence that BHR may help to identify subjects with rhinitis who are at risk for asthma progression (6, 7). In addition, a number of studies suggest that allergic rhinitis may prelude to airway symptoms related to asthma (8, 9).

Allergen-specific immunotherapy (SIT) is a widely used form of therapy for allergic conditions (10). Although the evidence of its effectiveness in asthma remains controversial (11), its efficacy in reducing the severity of symptoms related to allergic rhino-conjunctivitis is well-established (10, 12). It has also been suggested that when given prophylactically to susceptible individual SIT may be effective in preventing progression to asthma rather than reversing its course once the disease is established (13–15). The reasons for these beneficial effects are not known.

We have conducted a randomized, placebo-controlled 3-year study of SIT in nonasthmatic rhinitic subjects

monosensitized to *Parietaria* pollen with documented seasonal increases in nonspecific BHR and sputum eosinophils during the pollen season. Outcome measures included seasonal symptoms, use of rescue medication, BHR to methacholine, and eosinophilic airway inflammation in sputum. Possible progression of allergic rhinitis to asthma in both treatment groups was also assessed.

Methods

Study design

The present study took the form of a parallel group, double-blind, placebo-controlled trial of 3 year duration (December 1997–December 2000), during which we investigated the effects of SIT with *Parietaria judaica* extract, on (i) symptoms and medication score, (ii) airway methacholine responsiveness, (iii) eosinophil counts in the sputum, and (iv) the clinical course of atopy, in a group of rhinitic subjects allergic to *Parietaria*.

Upon entry of subjects into the study (December 1997 – out of *Parietaria* season), a detailed history 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 (increasing doses of allergen extract) or placebo. In 1998, 1999, and 2000 participants recorded daily symptoms and rescue medication on diary cards from March to July. 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.

Subjects

Thirty nonsmoking subjects (15 female and 15 male; 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 (Istituto Malattie Apparato Respiratorio, University of Catania, Sicily) (Table 1). These subjects were selected on the basis of positive skin prick tests (wheal > 3 mm) to *P. judaica* and negative tests to a panel of 21 common aeroallergens. Exclusion criteria were a past or present history of asthma, previous asthma symptoms or asthma medication intake, and/or abnormal spirometric values.

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 three consecutive years (1998–2000). The incidence of upper and/or lower airways symptoms and the use of rescue medications were scored and totaled daily. During the course of the study, the subjects were requested not to take nasal and oral corticosteroids.

Measurement of airway responsiveness The BHR was evaluated by methacholine bronchial challenge, as described previously (5). The bronchial responses to the inhaled methacholine were expressed as the provocative concentration causing a 15% decline in FEV₁ (PC₁₅), which was calculated by linear interpolation from the concentration–response curve constructed on a logarithmic scale.

Sputum induction and processing Induction was carried out according to our previously published method (5). Sputum plugs were solubilized by adding an equal weight of 0.01 M dithioerythritol (DTE; Fluka, Gillingham, Dorset, UK). Specimens were then vortexed, filtered and centrifuged at 400 g for 10 min at 4°C. The cell pellets were resuspended in 1 ml of phosphate-buffered saline (PBS) without Ca²⁺ and Mg²⁺ and viable cells counted in a hemocytometer. Differential counting was carried out on 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 number of total cells and as absolute counts.

Specific immunotherapy

A standardized extract of *P. judaica* (Alutard SQ, ALK-Abellò, Milan, Italy) was administered in a 'cluster' fashion between December 1997 and February 1998 followed by monthly maintenance injections until December 2000. Placebo injections of identical appearance contained 0.01 mg/ml of histamine acid phosphate in allergen diluent (PBS).

In most of the participants a maintenance dose of 80 000 standard quality units (SQ-U) was achieved. Each 0.8-ml maintenance

injection of 80 000 SQ-U was equivalent to 8000 biological units and contained 4.8 µg of the major allergen *Par J1*.

Statistical analyses

Symptoms and medication scores were summed up for the 8 weeks corresponding to the peak pollen counts each year and expressed as the area under the curve (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 nonparametric data (AUC values and sputum variables), differences between groups were compared using Kruskal–Wallis one-way ANOVA followed by Mann–Whitney *U*-test, whereas within-groups comparisons were analysed using Friedman's test followed by Wilcoxon's matched pairs signed rank test where appropriate. Logarithmically transformed data (PC₁₅ values) were analysed by using two-way ANOVA followed by the paired Student's *t*-test. Bonferroni's correction was applied to allow for multiple comparisons.

Fisher's Exact test was used to compare the frequency of development of asthma in the two study groups. Relationships between asthma progression and PC₁₅ values or percentage sputum eosinophils at the beginning of the study were analysed using independent samples *t*-test.

Results

Twenty-nine of the 30 participants completed the 3-year study period. The subject groups were comparable with respect to age, airway responsiveness to methacholine, severity of symptoms and sputum eosinophilia (Table 1). The treatment was well-tolerated, and no systemic reactions occurred throughout the study.

Effects of SIT on symptom and medication scores and on asthma Progression

By the third year of the study, a significant difference in symptom scores was observed between groups; the

Table 1. Clinical characteristics of participants

	<i>Parietaria</i> -SIT	Placebo
Number of participants	15	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 symptom 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.

* Scores derived at the peak of the *Parietaria* pollen season 1998 and expressed as the area under the curve (AUC).

† PC₁₅ methacholine <16 mg/ml.

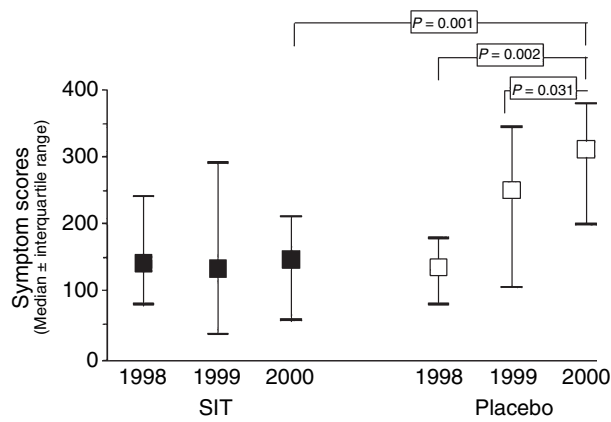


Figure 1. Changes in median ($\pm$ interquartile range) symptom scores in specific immunotherapy (SIT – closed squares) and placebo-treated (open squares) group at the peak of *Parietaria* seasons in 1998, 1999, and 2000. A significant difference ($P = 0.001$) was observed by the third year of treatment.

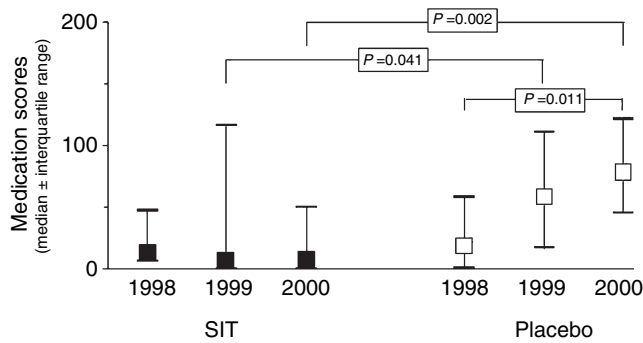


Figure 2. Changes in median ($\pm$ interquartile range) medication scores in specific immunotherapy (SIT – closed squares) and placebo-treated (open squares) group at the peak of *Parietaria* seasons in 1998, 1999, and 2000. Significant difference was observed in the second ($P = 0.041$) and third year ($P = 0.002$) of treatment.

median (interquartile range) symptom scores of 310 (198–387) in the placebo group was reduced to 145 (55–210) in the SIT group ($P = 0.001$) (Fig. 1). Comparison of 1998 with 2000 data, shows a significant change in overall median symptom scores by 121% (15–280) in the placebo group and –16% (–64 to 37) in the SIT group ($P = 0.001$). Similarly, a significant difference in medication scores was observed between groups, the greatest effect being observed by the third year of the study (Fig. 2).

Time-dependent progression of asthmatic symptoms and antiasthma medication scores was apparent in subjects with seasonal allergic rhinitis. By the end of the investigation, a total of nine of 29 participants developed symptoms compatible with the diagnosis of asthma; of these, seven (47%) belonged to the placebo group, whereas only two (14%) to the SIT-treated group ($P = 0.056$) (Fig. 3).

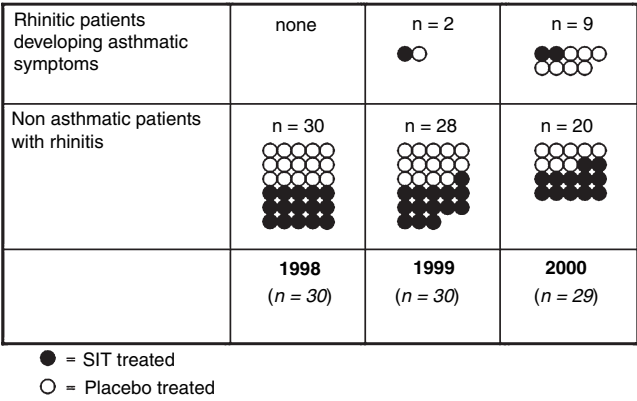
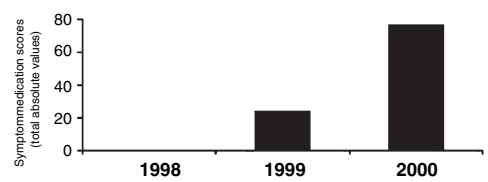


Figure 3. Upper panel: time-dependent progression of asthmatic symptoms and antiasthma medication intake in subjects with seasonal allergic rhinitis. Lower panel: progression to asthma of individual rhinitic subjects (circles) over the 3-year study period. Specific immunotherapy (SIT) is likely to prevent development of asthma. Nine of 29 participants developed asthmatic symptoms; of these, seven (47%) in the placebo group, whereas only two (14%) in the SIT-treated group. SIT- and placebo-treated subjects are shown as closed and open circles respectively.

Effects of SIT on methacholine airways responsiveness and on sputum eosinophils

Between group comparisons throughout the study showed no significant difference in PC_{15} methacholine. Likewise, no significant changes in PC_{15} methacholine values were observed within the placebo- and the SIT-treated group. Moreover, between- and within-group comparisons throughout the study showed no significant difference in percentage eosinophils counts.

Further analyses revealed that sputum eosinophils ($P = 0.014$) but not methacholine PC_{15} ($P = 0.108$) values at the beginning of the study is a good predictor of subsequent asthma progression.

Discussion

We have shown that SIT is effective at controlling symptoms and medication scores in allergic rhinitic subjects sensitized to *Parietaria* pollen. Differences in symptoms and medication scores between SIT- and placebo-treated subjects were already apparent by the first year of treatment. However, this improvement in clinical symptoms and medication scores was not associated with decline in BHR to methacholine or with modifications in sputum eosinophilia.

The most interesting finding in the present study is that nonasthmatic subjects with seasonal allergic rhinitis progress to bronchial asthma and SIT appears to prevent the natural course of the disease. Because of the small sample size, this effect of SIT did not reach significance ($P = 0.056$) but a trend was clearly apparent; 47% of subjects in the placebo group developed asthma symptoms by the end of the study, whereas in only 14% of those in the SIT group progression of allergic rhinitis to asthma was reported. These findings support the evidence that use of immunotherapy in nonasthmatic individual with rhinitis can reduce the occurrence of asthma (14, 16, 17). That, this might be the case, it has been suggested in a retrospective survey of nonasthmatic subjects with allergic rhinitis where subjects receiving SIT had 40% less chance of developing asthma compared with untreated subjects (18).

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 subsequent development of asthma in rhinitic individuals, 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 (*Parietaria* pollen season in Sicily ranges from February to October) is often responsible for almost perennial symptoms (19). As in previous studies with nonasthmatic subjects with allergic rhinitis (4, 5, 20), we have observed a substantial number of sputum eosinophils and elevated BHR during periods of seasonal exposure to *Parietaria* pollen, which is known to reflect active allergic inflammation of the airways. Moreover, in a multivariate analysis we have demonstrated that sensitization to *Parietaria* (but not house dust mite) is a significant independent risk factor for worsening and progression of upper and lower airways symptoms (18). 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 observed lack of effect of *Parietaria*-SIT against BHR to methacholine and sputum eosinophils is in agreement with earlier findings in subjects with seasonal asthma and rhinitis (21). However, our results are somewhat at variance with previous studies on mite, birch and grass pollen allergy (17, 22–24). The reasons for this discrepant effect of SIT on BHR and sputum eosinophilia are not quite clear, but 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 season. 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 airways responsiveness to methacholine and sputum eosinophilia.

This study shows that the beneficial effect of *Parietaria*-SIT containing symptoms and medications scores are not related to changes in BHR to methacholine or sputum eosinophilia. Subjects with seasonal allergic rhinitis progress to bronchial asthma and SIT may prevent the natural course of the disease. Our findings indicates that SIT should be considered earlier in the treatment of rhinitis in order to prevent progression to asthma, but larger studies are needed to confirm our observations and to define the characteristics of the patients who would benefit most from such therapeutic approach.

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