

Effects of Specific Immunotherapy in Allergic Rhinitic Individuals with Bronchial Hyperresponsiveness

ROSA D. GREMBIALE, LUIGI CAMPOROTA, SAVERIO NATY, CARMELINDO M. E. TRANFA, RATKO DJUKANOVIC, and SERAFINO A. MARSICO

Department of Experimental and Clinical Medicine, University of Catanzaro, Catanzaro, Italy; and Division of University Medicine, Southampton General Hospital, Southampton, United Kingdom

Allergic rhinitis can be associated with bronchial hyperresponsiveness (BHR), and carries an increased risk for the development of asthma. The aim of this study was to evaluate the ability of specific immunotherapy (SIT) to reduce the progression of allergic rhinitis to asthma and prevent the associated increase in BHR. Forty-four subjects monosensitized to *Dermatophagoides pteronyssinus*, with perennial rhinitis and BHR to methacholine, were randomly assigned to receive SIT or placebo in a double-blind study conducted over a period of 2 yr. After 1 yr of treatment, a 2.88-fold increase in the provocative dose of methacholine producing a 20% decrease in FEV₁ (PD₂₀FEV₁) was recorded in the SIT-treated group (95% confidence interval [CI]: 3.98- to 2.09-fold; $p < 0.001$), with a further increase to fourfold at the end of Year 2 (95% CI: 2.9- to 5.7-fold; $p < 0.001$). At the end of the study, the methacholine PD₂₀FEV₁ was within the normal range in 50% of treated subjects ($p < 0.0001$), and was significantly higher in this group than in the group receiving placebo ($p < 0.0001$). In contrast, no changes in methacholine PD₂₀FEV₁ were found in the placebo group throughout the study. Although 9% of subjects given placebo developed asthma, none of those treated with SIT did. This study suggests that SIT, when administered to carefully selected, monosensitized patients with perennial allergic rhinitis, reduces airway responsiveness in subjects with rhinitis, and may be an appropriate prophylactic treatment for rhinitic patients with hyperreactive airways.

Allergic rhinitis is one of the most common chronic diseases of both children and adults, and is often associated with other atopic diseases such as asthma and atopic dermatitis. Bronchial hyperresponsiveness (BHR) is considered a hallmark of bronchial asthma, and has been shown to precede the development of asthma in children as well as in adults (1, 2). Evidence from population studies suggests that airway hyperresponsiveness is associated with an accelerated decline in lung function (3) and with respiratory symptoms (4). It is also recognized that in a proportion of allergic rhinitic individuals, bronchial challenge with histamine or methacholine may reveal BHR in the absence of any lower airway symptoms (5). Recent studies have suggested that individuals who have allergic rhinitis are at increased risk for developing asthma (6), and a long-term follow-up study of atopic individuals has shown that about 10% of allergic rhinitic patients develop asthma, as compared with 3.6% of those with no prior history of rhinitis, and that a further 34 to 50% complain of airway symptoms related to asthma (7).

The increased risk for asthma developing in rhinitic patients is believed to be related to the presence of atopy, as defined by skin test reactivity to common aeroallergens, such that 32% of allergic rhinitic individuals have associated asthma,

as compared with 14% of nonallergic rhinitic individuals (8). In a recent 3-yr follow-up study of subjects with BHR to methacholine, further risks for the development of asthma were identified, including the presence of BHR, with the risk being related to the degree of responsiveness, the existence of first-degree relatives with asthma, and a history of virus infection during the follow-up period (9). The factors that determine the development of asymptomatic hyperresponsiveness are unclear, but conceivably involve genetic susceptibility and sensitization to different types of allergens.

Specific immunotherapy (SIT) is a widely used form of therapy for allergic diseases. Well-controlled clinical trials have demonstrated its efficacy in reducing the severity of symptoms related to allergic rhinitis and conjunctivitis (10). The evidence of its effectiveness in asthma is still controversial (11), possibly because of the multifactorial nature of chronic asthma and development of the structural changes referred to as airways remodelling (12), which may not respond favorably to SIT. However, it is possible that when given prophylactically to susceptible individuals, immunotherapy may be effective in preventing the development of asthma rather than reversing its course once the disease is established (13, 14). To test the hypothesis that SIT can reduce BHR, we conducted a 2-yr, double-blind, placebo-controlled trial of SIT involving atopic rhinitic subjects sensitized to house dust mite. Although these patients had upper airways disease only, they were deemed to be at risk of developing asthma on the basis of existing BHR as determined by methacholine challenge.

METHODS

Study Design

This was a double-blind, placebo-controlled trial lasting 2 yr, in which the effects of SIT on BHR, serum IgE, and the clinical course of atopy were investigated in a group of allergic rhinitic volunteers. Upon entry of subjects into the study, a detailed history was taken and a physical examination, spirometry, and a bronchial challenge with methacholine were done. Subjects were then randomized to receive either active treatment, consisting of increasing doses of allergen extract given subcutaneously, followed by monthly maintenance treatment, or placebo. The clinical effects were evaluated over the entire period of the study, and all subjects underwent methacholine challenge after 1 yr and 2 yr of treatment.

The study was approved by the local ethics committee, and written informed consent was obtained from each subject or, in the case of minors, from their parents.

Subjects

Forty four subjects (23 female and 21 male; mean age: 19 yr, age range: 10 to 38 yr) with a documented history of atopic rhinitis were enrolled into the study (Table 1). All of the subjects were nonsmokers and had a history of perennial symptoms of rhinitis without seasonal variations. None reported any symptoms compatible with asthma, and all had normal lung function tests. All of the subjects had positive skin prick tests to the house dust mite, *Dermatophagoides pteronyssinus*, but had negative tests with a series of other common aeroallergens (trees, grass pollen, dog hair, cat, *Olea europaea*, *Parietaria officinalis*

(Received in original form September 21, 1999 and in revised form July 14, 2000)

Correspondence and requests for reprints should be addressed to Luigi Camporota, Southampton General Hospital, University Medicine, Level D Centre Block, Tremona Road, SO16 6YD Southampton, UK. E-mail: Lc1@soton.ac.uk

Am J Respir Crit Care Med Vol 162, pp 2048-2052, 2000
Internet address: www.atsjournals.org

and *P. judaica*, *Aspergillus fumigatus*, *Alternaria*, *Candida albicans*). Sensitization to house dust mite was confirmed by the finding of increased titers of specific IgE antibodies to this allergen.

Study Outcome Variables

Measurement of airway responsiveness. FEV₁, FVC, and forced expiratory flow from 25% to 75% of FVC (FEF₂₅₋₇₅) were measured with a spirometer (MasterLab; Erich Jaeger GmbH, Hoechberg, Germany), and the results were expressed as percents of predicted values. Airway responsiveness was measured by methacholine bronchial challenge, using a method modified of Balzano and colleagues (15). Methacholine (Lofarma, Milan, Italy) was dissolved in physiologic saline to give 0.2% and 1% solutions, and was delivered via a dosimeter (Mefar MB3; Bovezzo [BS], Italy) that produces aerosol particles ranging from 0.5 to 5 μm in diameter. Inhalations were conducted with the subject in the sitting position and wearing a noseclip. After five breaths of aerosolized phosphate buffered saline (PBS) solution, subjects inhaled 1, 2, 4, and 8 breaths of the 0.2% solution of methacholine (equivalent to 0.1 μmol, 0.2 μmol, 0.4 μmol, and 0.8 μmol of methacholine), followed by 3, 6, and 12 breaths of the 1% solution (equivalent to 1.5, 2, and 6 μmol of methacholine). Spirometry was performed at 1 min and 3 min after each dose, and the challenge was stopped when a decrease of > 20% in FEV₁ had been achieved or when the maximum concentration of methacholine had been inhaled. The dose of methacholine was then plotted against the percent decrease in FEV₁ on a logarithmic scale, and the provocative dose causing a 20% decline in FEV₁ (PD₂₀FEV₁) calculated by linear interpolation. PD₂₀FEV₁ values ≥ 8 μmol of methacholine were accepted as indicating normal airway responsiveness (16).

Assessment of disease activity. All subjects visited the study center monthly. During the course of the study, the subjects were requested not to take topical decongestants, corticosteroids, or cromolyn, but were allowed to self-administer oral antihistamines for symptomatic relief. The number of tablets taken was recorded on a diary card. To follow symptoms throughout the 2 yr of the study, we chose not to use the rhinitis symptom score as an index of disease severity. Instead, subjects were invited to seek medical attention whenever they felt it necessary because of their clinical condition. The number of requests for medical attention (unscheduled visits) was used as an index of disease activity. In order to maintain the double blind protocol of the study, the physicians who consulted the subjects were not involved in the study.

Measurement of serum IgE levels. Serum IgE was measured at baseline and after Years 1 and 2 of treatment, using an enzyme-linked immunosorbent assay (Pharmacia, Uppsala, Sweden).

Immunotherapy. House dust mite allergen extract conjugated with sodium alginate (Conjuvac; Bayer, Milan, Italy) was used as SIT; the placebo used in the study, consisted of 10 mg/ml of histamine phosphate in physiologic 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. Immunotherapy was given according to the manufacturer's instructions. The starting dose of allergen, of 1 biologic unit (BU), was doubled on a weekly basis to a final dose of 800 BU or to the maximal dose tolerated (the dose that did not cause any systemic adverse effects, such as rhinitis or asthma, or swelling and erythema ≥ 10 cm in diameter). Once achieved, the maintenance dose was repeated monthly for the entire duration of the study. After each injection, subjects were kept under close observation for 30 min, and at their next visit were asked about any late side effects.

Statistical Analyses

The PD₂₀FEV₁ and IgE data collected in the study were transformed logarithmically and presented as geometric means and ranges. The effect of treatment on BHR and serum IgE was analyzed with logarithmically transformed data, using two-way analysis of variance followed by the paired Student's *t* test. Bonferroni's correction was applied to allow for multiple comparisons.

Changes in bronchial responsiveness at the end of the second year were calculated as the antilogarithm of the difference between log₁₀ PD₂₀FEV₁ at the end of the second year and log₁₀ PD₂₀FEV₁ at baseline, and were expressed as units of fold difference in PD₂₀FEV₁ with a 95% confidence interval (CI) (17). Correlations between levels of serum IgE at baseline and units of fold difference in PD₂₀FEV₁ were measured with Spearman's correlation test. The relation between PD₂₀FEV₁ at baseline and the change in PD₂₀FEV₁ over the 2-yr study period was examined by correlating the average of the initial and final measurements ((PD₂₀FEV₁ baseline + PD₂₀FEV₁ second year)/2) with the observed change (PD₂₀FEV₁ second year - PD₂₀FEV₁ baseline) (18). Fisher's exact test was used to compare the frequency of asthma in the two study groups.

RESULTS

The treatment was well tolerated, and no systemic reactions occurred during the study.

TABLE 1
PATIENT CHARACTERISTICS

	Placebo	SIT
Subjects, n	22	22
Age, yr*	18 (10-38)	19 (10-35)
Sex	12 F; 10 M	11 F, 11 M
Height, cm*	161.4 (160-163)	163.67 (157-171)
FEV ₁ , %pred†	107.6 ± 9.1	110.27 ± 7.42
PD ₂₀ FEV ₁ , μg‡	245.5 (95% CI: 213.8-281.8)	89.1 (95% CI: 77.6-102.8)
PD ₂₀ FEV ₁ first year, μg‡	269.1 (95% CI: 234.4-309)	630.9 (95% CI: 426.6-933.2)
PD ₂₀ FEV ₁ second year, μg‡	288.4 (95% CI: 263-316.2)	758.6 (95% CI: 562.3-1000)
IgE baseline, IU/L†	336.6 ± 253.9	458.95 ± 357.3
IgE first year, IU/L†	328.2 ± 238.4	450.91 ± 290.9
IgE second year, IU/L†	328.8 ± 239.4	399.82 ± 333.09
Drug intake first year, tablets	NR	NR
Drug intake second year, tablets	120	52
No. of unscheduled visits, first year	8	7
No. of unscheduled visits, second year	10	0

Definition of abbreviations: CI = confidence interval; PD₂₀FEV₁ = provocative dose of methacholine needed to reduce FEV₁ by 20%; SIT = specific immunotherapy.

* Data are shown as arithmetic mean (range).

† Data are shown as arithmetic mean ± SD.

‡ Data are shown as geometric mean (95% CI).

Effects on Airways Responsiveness to Methacholine

At baseline, the two groups of subjects were well matched, with no significant differences in methacholine PD₂₀FEV₁ ($p = 0.15$) (Table 1). After the first year of treatment, a significant increase in methacholine PD₂₀FEV₁ was found in the SIT-treated group (mean: 2.88-fold, 95% CI: 3.98- to 2.09-fold; $p < 0.001$). There was a further increase in PD₂₀FEV₁ at the end of the second year of treatment, as compared either with pre-treatment values (mean: 4.1-fold difference; 95% CI: 2.9- to 5.7-fold; $p < 0.001$) or with values after the first year of treatment ($p = 0.001$) (Figure 1; Table 1). In contrast, no differences in PD₂₀FEV₁ were found in the placebo group throughout the study ($p = 0.708$).

Although there was no difference between the two groups in methacholine responsiveness at the beginning of the study, there was a significant difference in PD₂₀FEV₁ after 2 yr of treatment ($p < 0.0001$). Fifty percent of the subjects receiving SIT had an increase in PD₂₀FEV₁ to $> 8 \mu\text{mol}$ of methacholine, whereas all of the patients given placebo continued to have BHR.

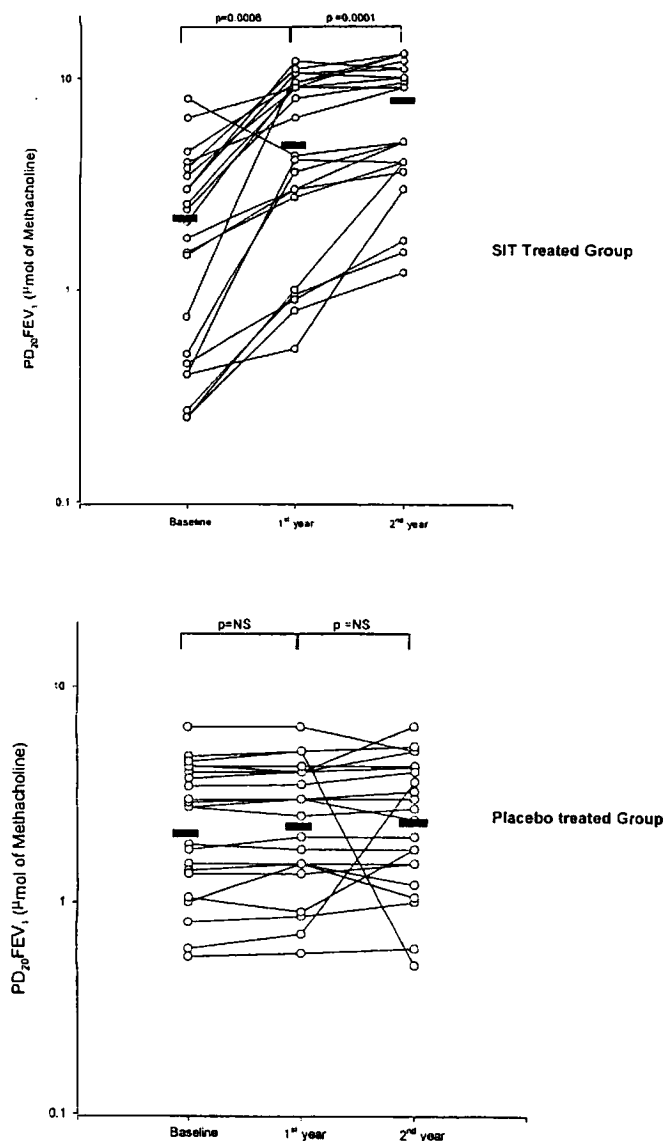


Figure 1. Measurement of BHR to methacholine (PD₂₀FEV₁) in the SIT-treated group at baseline and after each year of study. After the first year of treatment there was a 2.88-fold increase in geometric mean PD₂₀FEV₁ ($p < 0.001$), which further increased to more than fourfold at the end of the second year of treatment ($p < 0.001$)

In the subjects treated with SIT, there was a significant positive correlation between PD₂₀FEV₁ at baseline and the magnitude of change in PD₂₀FEV₁ at the end of the study ($r = 0.75$, $p < 0.0001$), as calculated by taking the average of the initial and final measurements in PD₂₀FEV₁ ($[\text{PD}_{20}\text{FEV}_1 \text{ baseline} + \text{PD}_{20}\text{FEV}_1 \text{ second year}]/2$) and correlating it with the observed change in PD₂₀FEV₁ at the end of the second year ($\text{PD}_{20}\text{FEV}_1 \text{ second year} - \text{PD}_{20}\text{FEV}_1 \text{ baseline}$) (Figure 2).

Disease Severity Score

Subjects treated with SIT visited the study center less frequently because of worsening of symptoms than did those given placebo. During the first year, control subjects made an average of eight unscheduled visits, as compared with seven visits for the SIT group, whereas during the second year the SIT group made no additional visits, compared with 10 visits for the control subjects. The average number of antihistamine tablets taken during the second year of the study was 52 in the SIT group versus 120 in the placebo group (Table 1). At the end of the second year, none of the SIT-treated subjects had developed asthma, whereas 9% of subjects in the placebo group reported symptoms compatible with mild asthma ($p = 0.49$).

Serum IgE Levels

Serum IgE concentrations did not differ in the SIT and placebo groups at baseline ($p = 0.50$), and did not change significantly during the study in either the SIT-treated subjects or those receiving placebo (Table 1). Within the group of SIT-treated subjects, serum IgE levels did not correlate with the change in methacholine PD₂₀FEV₁ (expressed in units of fold difference in PD₂₀FEV₁) from baseline to the end of the second year of the study ($r = -0.25$; $p = 0.267$).

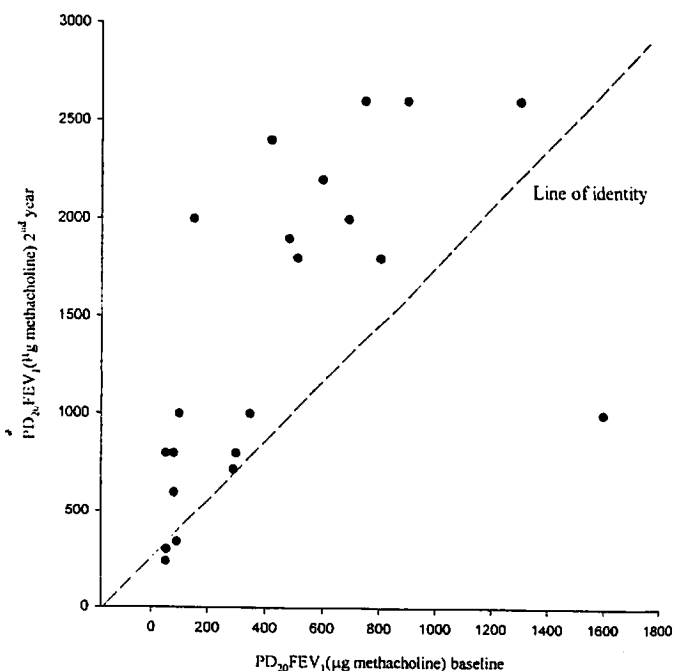


Figure 2. Correlation between PD₂₀FEV₁ at baseline and the magnitude of change in PD₂₀FEV₁ at the end of the study. The correlation was calculated by taking the average of the initial and final measurements of PD₂₀FEV₁ ($[\text{PD}_{20}\text{FEV}_1 \text{ baseline} + \text{PD}_{20}\text{FEV}_1 \text{ second year}]/2$) and correlating it with the observed change in PD₂₀FEV₁ at the end of the second year ($\text{PD}_{20}\text{FEV}_1 \text{ second year} - \text{PD}_{20}\text{FEV}_1 \text{ baseline}$). The broken line represents the line of identity, and the distance between any single observation and the identity line represents the change in PD₂₀FEV₁.

DISCUSSION

Our study suggests that SIT can reduce BHR in hyperresponsive patients with perennial allergic rhinitis who do not have any symptoms of asthma. This pilot study, although not designed to detect differences in the incidence of asthma between the SIT and placebo study groups, suggests that individuals with allergic rhinitis should undergo testing of their bronchial reactivity in order to identify those with BHR and to provide a window of opportunity in which SIT may be effective at preventing progression to asthma. The finding of a positive correlation between the methacholine $PD_{20}FEV_1$ before the commencement of SIT and the magnitude of improvement in bronchial reactivity suggests that early intervention, when bronchial reactivity is relatively mild, is likely to be of greater benefit, possibly because of less advanced airways remodeling. These findings, in an adult population of allergic rhinitic individuals sensitized to house dust mite, a perennial and strong determinant of asthma, support the finding in the preventive allergen treatment study, which involved children with allergic rhinitis who were sensitized to birch and/or timothy pollen, that SIT can reduce the occurrence of asthma (14).

The treatment of allergic diseases involves either the use of drugs (anti-histamines or corticosteroids) that block the production or action of pro-inflammatory mediators, and/or allergen-specific treatments, such as allergen avoidance and immunotherapy. The benefits of SIT in the treatment of respiratory allergic disease have been studied for both rhinitis (19) and asthma (20). That most individuals with atopic diseases are sensitized to more than one allergen, in addition to the risk of anaphylactic reactions occurring during SIT and the availability of effective topical anti-inflammatory drugs, has been used as an argument against using SIT in asthma (11) and in all but the most severe forms of rhinitis, in which antihistamines and topical medications insufficiently control symptoms (21). In the present study we chose to focus on BHR in the absence of lower respiratory tract symptoms as a target for prophylactic immunotherapy. Our hypothesis was based on convincing evidence that allergic rhinitis may be a prelude to overt asthma (1, 2). Furthermore, we restricted the inclusion criteria for the study to subjects who were sensitized to only one allergen: house dust mite. This choice was based on the broadly held view that early sensitization to this perennial allergen poses a greater risk for asthma (22).

The slow onset of action, the expenses involved, and the inconvenience to the patient are additional drawbacks of SIT. In this study, we noted a significant improvement in BHR by 1 yr after initiating SIT. Further improvement was observed at the end of the second year, when 50% of SIT-treated patients showed an increase in $PD_{20}FEV_1$ to levels within the normal range, whereas all of the control subjects continued to have BHR. Our primary aim was not to evaluate the clinical efficacy of SIT in rhinitis, since this issue had already been addressed in well-conducted controlled trials. Nevertheless, in keeping with these earlier studies (10), we noted a positive effect of SIT on symptoms of rhinitis as well as on the need for relief medication, as reflected in fewer unscheduled consultations.

Our study also shows that rhinitic patients can have persistent BHR even in the absence of clear symptoms of asthma or other lower respiratory symptoms. The full clinical relevance of asymptomatic BHR remains unclear. In accord with other open studies (6), we found that by the end of our study, nearly 10% of patients receiving placebo developed asthma. Longitudinal population studies have shown a faster decline of pulmonary function in individuals with BHR than in those with normal bronchial responsiveness (23), with the severity of BHR

correlating positively with the degree of decline (24). In a previously reported 2-yr follow-up study, about 20% of subjects with asymptomatic BHR developed asthma, as compared with 2% of subjects without BHR (25). In another longitudinal study, lasting 6 yr, the percentage of subjects with BHR developing asthma was as high as 58%, but the prevalence of asthma in subjects without BHR also increased, to 13% (26).

It is unclear why a proportion of individuals with atopy and rhinitis eventually develop asthma, or what determines the rate of conversion. Although atopy *per se* carries an increased risk for asthma developing in rhinitic individuals, chronic exposure to perennial allergens, and particularly indoor allergens, is more important than seasonal exposure to such allergens as pollens. The presence of asthma among first-degree relatives of patients whose rhinitis is complicated by asthma (27) suggests possible inheritance of factors that determine BHR, independent of atopy. Persons with asthma often develop first symptoms after a respiratory infection, and this also appears to be a risk factor for asthma in patients with BHR (27).

One reason for the poor understanding of the natural history of respiratory allergic disorders is the lack of sufficient insight into the lower airway pathology of individuals who have rhinitis only. Limited studies of bronchial biopsy specimens from such patients have shown increased numbers of mucosal eosinophils and an increased thickness of subepithelial collagen, although both are generally present to a lesser degree than is observed in asthma (28). However, it is unclear how this relates to BHR and the subsequent development of asthma, although some studies have shown a correlation between the degree of airway eosinophilia and subepithelial fibrosis and BHR (29).

In the absence of information about any cellular changes in the airways of patients participating in the present study, we are unable to offer an explanation for the protective effect of SIT observed in the study. Even though SIT has long been used, its mechanisms of action are far from clear. Among the different hypotheses for its effects, earlier studies have focused on its effect on circulating antibodies, such as a reduction in IgE levels or an increased production of allergen-blocking IgG antibodies. The protective effect of the increased levels of allergen-specific IgG found after SIT (the "blocking antibody" theory) is still debated, and is viewed by some authors as a "bystander" event (21). Our study failed to show a relevant effect of SIT on IgE levels. Furthermore, neither total nor specific serum IgE levels were correlated with methacholine $PD_{20}FEV_1$. These findings are in accord with those in previous studies (30). Recent evidence suggests that SIT alters T-cell responses to allergen stimulation, increasing the proportion of T cells expressing a T-helper type 1 phenotype (31), or of T cells expressing messenger RNA for interferon (IFN)- γ in association with an increased production of interleukin (IL)-12-positive cells (32). This effect might provide a basis for inhibition of both the early and late responses seen after immunotherapy (33). In accord with this explanation is that other studies have reported a reduced production of IL-4 (34) and IL-5 (35) with SIT, whereas conflicting data are available for IFN- γ production (35, 36). One hypothesis, therefore, is that SIT might target the mild but persistent bronchial inflammation in rhinitic individuals with BHR.

As with other diseases, prevention of asthma offers great benefits. Any progress in this respect is hampered by a lack of better understanding of the early pathogenesis of clinical asthma. There is little or no evidence that early treatment with corticosteroids might be appropriate in this setting. One advantage of SIT as compared with other forms of treatment might be its ability to modify airway sensitivity to environmen-

tal stimuli in an allergen-specific manner, despite greater cost and potential risk. Our study provides encouraging evidence that SIT is an effective prophylactic treatment for rhinitic individuals in whom BHR can be demonstrated. However, further and larger studies are needed to confirm our observations and to define the characteristics of the patients who would benefit most from such therapy. Only then would it be appropriate to recommend what should be done.

References

- Sparrow D, O'Connor G, Colton T, Barry CL, Weiss ST. The relationship of nonspecific bronchial responsiveness to the occurrence of respiratory symptoms and decreased levels of pulmonary function: the Normative Aging Study. *Am Rev Respir Dis* 1987;135:1255-1260.
- Hopp RJ, Townley RG, Biven RE, Bewtra AK, Nair NM. The presence of airway reactivity before the development of asthma. *Am Rev Respir Dis* 1990;141:2-8.
- Gottlieb DJ, Sparrow D, O'Connor GT, Weiss ST. Skin test reactivity to common aeroallergens and decline of lung function: the Normative Aging Study. *Am J Respir Crit Care Med* 1996;153:561-566.
- Gerritsen J, Koeter GH, Postma DS, Schouten JP, Knol K. Prognosis of asthma from childhood to adulthood. *Am Rev Respir Dis* 1989;140:1325-1330.
- Ramsdale EH, Morris MM, Roberts RS, Hargreave FE. Asymptomatic bronchial hyperresponsiveness in rhinitis. *J Allergy Clin Immunol* 1985;75:573-577.
- Greisner WA, Settignano RJ, Settignano GA. Co-existence of asthma and allergic rhinitis: a 23-year follow-up study of college students. *Allergy Asthma Proc* 1998;19:185-188.
- Danielsson J, Jessen M. The natural course of allergic rhinitis during 12 years of follow-up. *Allergy* 1997;52:331-334.
- Wright AL, Holberg CJ, Martinez FD, Halonen M, Morgan W, Taussig LM. Epidemiology of physician-diagnosed allergic rhinitis in childhood. *Pediatrics* 1994;94(6 Pt 1):895-901.
- Laprise C, Boulet LP. Asymptomatic airway hyperresponsiveness: a three-year follow-up. *Am J Respir Crit Care Med* 1997;156(2 Pt 1):403-409.
- Malling H. Position paper: immunotherapy. The European Academy of Allergy and Clinical Immunology (EAACI). *Allergy* 1993;48:7-35.
- Barnes PJ. Is immunotherapy for asthma worthwhile? *N Engl J Med* 1996;334:531-532.
- Busse W, Elias J, Sheppard D, Banks-Schlegel S. Airway remodeling and repair. *Am J Respir Crit Care Med* 1999;160:1035-1042.
- Jacobsen L, Nuchel Petersen B, Wihl JA, Lowenstein H, Ipsen H. Immunotherapy with partially purified and standardized tree pollen extracts: IV. Results from long-term (6-year) follow-up. *Allergy* 1997;52:914-920.
- Valovirta E. Capacity of specific immunotherapy in prevention of allergic asthma in children: the Preventive Allergy Treatment Study (PAT). *J Invest Allergol Clin Immunol* 1997;7:369-370.
- Balzano G, Delli Carri I, Gallo C, Cocco G, Melillo G. Intrasubject between-day variability of PD20 methacholine assessed by the dosimeter inhalation test. *Chest* 1989;95:1239-1243.
- Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD, Juniper EF, Malo JL. Airway responsiveness: standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report of the Working Party on Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl* 1993;16:53-83.
- Peat JK, Unger WR, Combe D. Measuring changes in logarithmic data, with special reference to bronchial responsiveness. *J Clin Epidemiol* 1994;47:1099-1108.
- Altman D. Practical statistics for medical research. London: Chapman & Hall; 1991. p. 278-288.
- Corrado OJ, Pastorello E, Ollier S, Cresswell L, Zanussi C, Ortolani C, Incorvaia A, Fugazza A, Lovely JR, Harris RI, et al. A double-blind study of hyposensitization with an alginate conjugated extract of *D. pteronyssinus* (Conjuvac) in patients with perennial rhinitis: 1. Clinical aspects. *Allergy* 1989;44:108-115.
- Abramson MJ, Puy RM, Weiner JM. Is allergen immunotherapy effective in asthma? A meta-analysis of randomized controlled trials. *Am J Respir Crit Care Med* 1995;151:969-974.
- Bousquet J, Lockey R, Malling HJ, Alvarez-Cuesta E, Canonica GW, Chapman MD, Creticos PJ, Dayer JM, Durham SR, Demoly P, Goldstein RJ, Ishikawa T, Ito K, Kraft D, Lambert PH, Lowenstein H, Muller U, Norman PS, Reisman RE, Valenta R, Valovirta E, Yssel H. Allergen immunotherapy: therapeutic vaccines for allergic diseases. World Health Organization. American Academy of Allergy, Asthma and Immunology. *Ann Allergy Asthma Immunol* 1998;81(5 Pt 1):401-405.
- Aberg N. Birth season variation in asthma and allergic rhinitis. *Clin Exp Allergy* 1989;19:643-648.
- O'Connor GT, Sparrow D, Weiss ST. A prospective longitudinal study of methacholine airway responsiveness as a predictor of pulmonary-function decline: the Normative Aging Study. *Am J Respir Crit Care Med* 1995;152:87-92.
- Parker DR, O'Connor GT, Sparrow D, Segal MR, Weiss ST. The relationship of nonspecific airway responsiveness and atopy to the rate of decline of lung function: the Normative Aging Study. *Am Rev Respir Dis* 1990;141:589-594.
- Zhong NS, Chen RC, Yang MO, Wu ZY, Zheng JP, Li YF. Is asymptomatic bronchial hyperresponsiveness an indication of potential asthma? A two-year follow-up of young students with bronchial hyperresponsiveness. *Chest* 1992;102:1104-1109.
- Jones A. Asymptomatic bronchial hyperreactivity and the development of asthma and other respiratory tract illnesses in children. *Thorax* 1994;49:757-761.
- Laprise C, Boulet LP. Airway responsiveness and atopy in families of patients with asthma. *Clin Invest Med* 1996;19:461-469.
- Djukanovic R, Lai CK, Wilson JW, Britten KM, Wilson SJ, Roche WR, Howarth PH, Holgate ST. Bronchial mucosal manifestations of atopy: a comparison of markers of inflammation between atopic asthmatics, atopic nonasthmatics and healthy controls. *Eur Respir J* 1992;5:538-544.
- Chetta A, Foresi A, Del Donno M, Consigli GF, Bertorelli G, Pesci A, Barbee RA, Olivieri D. Bronchial responsiveness to distilled water and methacholine and its relationship to inflammation and remodeling of the airways in asthma. *Am J Respir Crit Care Med* 1996;153:910-917.
- Annesi I, Oryszczyn MP, Frette C, Neukirch F, Orvoen-Frija E, Kauffmann F. Total circulating IgE and FEV₁ in adult men: an epidemiologic longitudinal study. *Chest* 1992;101:642-648.
- Durham SR, Varney V, Gaga M, Frew AJ, Jacobson M, Kay AB. Immunotherapy and allergic inflammation. *Clin Exp Allergy* 1991;21(Suppl 1):206-210.
- Hamid QA, Schotman E, Jacobson MR, Walker SM, Durham SR. Increases in IL-12 messenger RNA+ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J Allergy Clin Immunol* 1997;99:254-260.
- Varney VA, Hamid QA, Gaga M, Ying S, Jacobson M, Frew AJ, Kay AB, Durham SR. Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J Clin Invest* 1993;92:644-651.
- Secrist H, Chelen CJ, Wen Y, Marshall JD, Umetsu DT. Allergen immunotherapy decreases interleukin 4 production in CD4+ T cells from allergic individuals. *J Exp Med* 1993;178:2123-2130.
- Jutel M, Pichler WJ, Skrbic D, Urwyler A, Dahinden C, Muller UR. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN- γ secretion in specific allergen-stimulated T cell cultures. *J Immunol* 1995;154:4187-4194.
- O'Brien RM, Byron KA, Varigos GA, Thomas WR. House dust mite immunotherapy results in a decrease in Der p 2-specific IFN- γ and IL-4 expression by circulating T lymphocytes. *Clin Exp Allergy* 1997;27:46-51.