

Injection Immunotherapy with Different Airborne Allergens Did Not Prevent de novo Sensitization to Ragweed and Birch Pollen North of Milan

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Key Words

Respiratory allergy · Immunotherapy · Sensitization · Genetics · Prevention

Abstract

Background: Previous studies suggest that allergen-specific immunotherapy (SIT) with a single allergen may prevent sensitization to other airborne allergens. **Objective:** To assess the effectiveness of injection SIT in preventing sensitization to birch and ragweed pollen north of Milan, a geographical area that was recently colonized by these two airborne allergens. **Methods:** 691 adult patients monosensitized to grass (n = 342), pellitory (n = 76), ragweed (n = 66), birch (n = 112), and house dust mite (n = 95) were studied. 284 (41%) of them received injection SIT as part of routine outpatient care; the remaining 407 (59%) not submitted to SIT, served as controls. All patients underwent follow-up visits after no less than 2 years in order to detect possible new sensitization to birch and/or ragweed. **Results:** 227 (33%) patients became sensitized to birch and/or ragweed pollen during the follow-up period. Surprisingly, the prevalence of new sensitizations was significantly higher among subjects receiving SIT (132/284; 46%) than among those not receiving SIT (95/407; 23%; $p < 0.001$). 27 subjects developed new sensitization to birch and/or ragweed pollen

while undergoing SIT. These findings were consistent in all subgroups with different airborne allergies. **Conclusion:** In this study, injection SIT did not exert any preventive effect against denovo sensitization to the two novel airborne allergens, birch and ragweed pollen, in adult monosensitized patients.

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Introduction

The efficacy and long-term effect of injection allergen-specific immunotherapy (SIT) in reducing symptoms in patients with airborne allergies have been established by a number of studies [1–4]. In contrast, whether SIT with a single allergen may also exert a preventive effect against sensitization to different airborne allergens is less well documented. To date, only three studies addressed this issue. Two of them were carried out in children sensitized to house dust mite [5, 6] and one was a retrospective study performed in an adult population [7]. The conclusion of all three studies was that SIT with a single allergen prevents sensitization to other airborne allergens.

Generally, airborne allergens present in a certain geographic area remain rather constant; as a consequence, all individuals are exposed to all airborne allergens present in that area from early infancy, the only variable being expo-

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sure to animal dander. The effects of exposure of the general population to one or more new airborne allergens may represent a unique opportunity to investigate the relevance of individual predisposition in sensitization mechanisms. For instance, in the suburban area north of Milan, birches became very popular ornamental plants in 1970s and 1980s; this caused an increased appearance of a significant allergic sensitization to tree pollen throughout the 1980s, as reported by two studies carried out in the area of Milan at the beginning of the 1990s [8, 9]. A similar increase was also noted in a recent position paper by the European Academy of Allergology and Clinical Immunology [10]. More recently, the whole area north of Milan was colonized by short ragweed starting from the surroundings of the International Malpensa Airport. The first reports of ragweed allergy in that area appeared in 1989–1990 [11, 12]. Since then, ragweed has become the second most frequent cause of respiratory allergy north of Milan after grass pollen. The appearance of new ubiquitous, highly allergenic airborne allergens in a certain geographic area might represent an interesting opportunity to assess whether injection SIT with different airborne allergens is able to prevent sensitization to novel allergens. The present study aimed to investigate the effectiveness of injection SIT in preventing sensitization to ragweed and birch pollen in monosensitized patients with respiratory allergy living in the area north of Milan.

Patients and Methods

Patients

The study was carried out on patients receiving care at the allergy unit of Bollate hospital. Data from 691 adult patients (age > 12 years) monosensitized to the 5 main airborne allergens present in this area, namely grass (n = 342), pellitory (n = 76), ragweed (n = 66), birch (n = 112), and house dust mite (n = 95) first seen between January 1st, 1986, and December 31st, 1998 and reevaluated no less than 2 years after the first visit (or after the end of SIT) were retrospectively reviewed. Monosensitization was defined as the presence of a single positive skin prick test (SPT) among grass, pellitory, ragweed, mugwort, plantain, birch, olive, cypress, house dust mite, *Alternaria*, dog dander, and cat dander (Allergopharma, Reinbeck, Germany) in keeping with clinical history. Patients showing even only weak skin responses to allergens other than the one responsible for respiratory allergy were excluded. The follow-up visits consisted of a thorough interview to ascertain possible changes in clinical symptoms and in SPTs with a complete series of airborne allergens (see above). SPTs were carried out using commercial extracts produced by the same manufacturer on all occasions. New sensitization to birch and/or ragweed pollen was defined as the appearance of a positive skin response accompanied by typical clinical symptoms (seasonal rhinoconjunctivitis and/or asthma from the beginning of March up to the end of May for birch pollinosis, and from the beginning of August up to the

end of September for ragweed pollinosis). All patients, both receiving and not receiving SIT, were given adequate therapies to control their respiratory symptoms. Patients monosensitized to other airborne allergens, such as plantain, mugwort, cypress, or olive pollen, molds and animal dander, were not included in this study due to the small number of subjects or to the small number of patients receiving SIT.

Skin Tests

SPTs were carried out using disposable 1-mm-tip lancets (Dome Hollister/Stier). Readings were taken at 15 min. Histamine 10 mg/ml and saline were used as positive and negative control, respectively. Wheals whose mean diameter exceeded 3 mm were considered positive [13]; however, all patients included in this study showed skin reactivities exceeding the mean diameter of 7 mm on SPTs. All patients scored negative on SPTs with saline.

Specific Immunotherapy

Injection SIT was offered to all 691 patients; 284 [41%] of them pursued the treatment. Most of the remaining patients refused SIT because of lack of time, fear of adverse reactions, or fear of injections. Patients receiving and not receiving SIT did not show any difference in age, sex, or prevalence of asthma (about 35–40%), and did not seem to show any difference in baseline symptom severity, as assessed by the consumption of anti-allergic drugs. All patients were given the same treatments for allergic rhinitis (local corticosteroids plus oral anti-histamines on demand) and for asthma (local corticosteroids plus local β -agonists on demand) irrespective of SIT. One-hundred-sixty-nine, 29, 41, 9, and 36 patients underwent SIT with pollen extracts of grass, pellitory, birch, and ragweed, respectively, and 36 patients underwent SIT with house dust mite extract. Commercial depot aluminum hydroxide-adsorbed allergen extracts from different manufacturers (Allergopharma, Reinbeck, Germany; Dome-Hollister/Stier, Spokane, Wash., USA; or Lofarma, Milan, Italy) were employed for SIT. SIT was administered following a perennial schedule in all cases. In pollen-allergic patients, doses were reduced by half during the pollen season. Weekly doses were given during the 'build-up' (induction) phase in order to reach the planned maintenance dose that was 1.0 ml of the final vial. Based upon the standardization methods used by the different manufacturers, this dose corresponded to 5,000 UT (therapeutic units) in Allergopharma extracts, 10,000 PNU in DHS extracts, and 10,000 U in Lofarma extracts. In practice, the planned maintenance dose could not be achieved in all patients due to the appearance of generalized urticaria or rhinitis/asthma, and some subjects were therefore maintained on the highest tolerated dose that did not elicit systemic side effects. It has been proposed that this corresponds to the optimal dose for SIT [14]. Maintenance doses were given at 3-week intervals for at least 3 years. All SIT were administered by the author at the allergy center.

Statistical Methods

Since the age and follow-up duration data followed a normal distribution, means were compared by the two-tailed Student *t* test. Proportions were compared by the χ^2 test with Yates' correction. Probability values less than 5% were considered significant. Several patients developed new sensitization appearing during the first year of SIT. Since SIT given for such a short period is probably insufficient to confer any protection against the development on new sensitization, statistical analyses were repeated including these subjects in the non-SIT group.

Table 1. Analysis of the total study population

	No SIT	SIT	p
Total number of patients	407 (59%)	284 (41%)	
M/F (312/378)	177/230	135/148	NS
Age, mean $\pm$ SD, years	27.0 $\pm$ 12.6	27.0 $\pm$ 12.4	NS
Follow-up, mean $\pm$ SD, years	7.4 $\pm$ 3.9	8.9 $\pm$ 4.1	<0.001
Post-SIT follow-up, mean $\pm$ SD, years	–	5.0 $\pm$ 2.9	
New sensitizations by R or B	95 (23%)	132 (46%)	<0.001
New sensitizations during SIT	–	27	
New sensitizations (corrected) ¹	122/434 (28%)	105/257 (41%)	<0.01
New sensitizations to other allergens	15 (4%)	12 (4%)	NS

R = Ragweed; B = birch.

¹ Analysis repeated including 27 patients who developed their new sensitization during the first 12 months of SIT in the No-SIT group.

Table 2. Analysis of the subpopulations of allergic patients

Allergen Pts	SIT	M/F	Age mean $\pm$ SD years	Follow-up mean $\pm$ SD years	New sensitizations by R or B	New sensitizations during SIT	New sensitizations (corrected)
Grass 342	no	80/93	22.7 $\pm$ 10.4	8.0 $\pm$ 4.1	58 (34%)	–	74/189 (39%)
	yes	81/88	24.4 $\pm$ 11.1	9.3 $\pm$ 4.0**	91 (54%)*	16	81/153 (53%)
Pellitory 76	no	19/28	29.1 $\pm$ 9.6	7.9 $\pm$ 4.2	10 (21%)		–
	yes	12/17	31.8 $\pm$ 10.8	8.8 $\pm$ 3.8	12 (41%)	0	–
Birch 112	no	23/48	35.9 $\pm$ 10.8	7.0 $\pm$ 3.8	18 (25%)		27/80 (34%)
	yes	19/22	38.9 $\pm$ 9.0	7.0 $\pm$ 4.3	20 (49%)*	9	11/32 (34%)
Ragweed 66	no	24/33	36.5 $\pm$ 11.9	5.8 $\pm$ 3.1	6 (10%)		8/59 (13%)
	yes	4/5	40.7 $\pm$ 10.3	6.7 $\pm$ 2.7	2 (22%)	2	0/7 (0%)
Mite 95	no	31/28	18.0 $\pm$ 10.9	6.9 $\pm$ 3.7	3 (5%)		–
	yes	19/17	18.2 $\pm$ 9.5	10.3 $\pm$ 4.2***	7 (19%)	0	–

Legends as in figure 1.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Results

All patients included in this study complied with the injection SIT schedule for 3 years. Patients receiving and not receiving SIT are compared in table 1. A total of 227 (38%) patients developed de novo sensitization to ragweed and/or birch pollen during the follow-up period. Subjects in the SIT group showed a markedly higher prevalence of new sensitizations than the untreated group (46 vs. 23%, respectively; $p < 0.001$); this difference was still present after correction for 27 subjects who developed their new sensitization during the first 12 months of SIT (table 1). By contrast, de novo sensitization to ‘traditional’ airborne allergens (that is all but ragweed and birch

pollen) was rare and did not show any difference between patients receiving and not receiving SIT (4 vs. 4%, respectively; $p = \text{NS}$). No de novo sensitization to ‘traditional’ allergens occurred during SIT. The total follow-up duration (the time between the first visit and the last follow-up visit) was significantly longer in subjects receiving SIT than in those not receiving SIT ($p < 0.001$), whereas the mean post-SIT follow-up (the time between the end of SIT and the last control visit) was significantly shorter than the total follow-up period in patients not receiving SIT ($p < 0.001$). Follow-up duration was not associated with a higher prevalence of new sensitizations to birch or ragweed pollen (i.e., the likelihood of a new sensitization did not depend on the year of recruitment for the study; data

not shown). Patients receiving and not receiving SIT allergic to different airborne allergens are compared in table 2. The former consistently showed a higher prevalence of de novo sensitization to ragweed or birch pollen. The total follow-up time was longer in patients receiving SIT than in those not receiving SIT, whereas the mean post-SIT follow-up was significantly shorter than the follow-up duration in patients not receiving SIT ($p < 0.001$ in all subgroups, not shown in table). Among patients receiving SIT, no relationship between the top allergen dose administered and subsequent new sensitization was observed in any of the subgroups. SIT was successful in the large majority (93%) of treated subjects, and the tendency to develop new sensitization was in no way associated with a poor effect of SIT. The results were not influenced by the use of allergenic extracts by different manufacturers.

Discussion

A recent study carried out in this geographic area found that subjects becoming monosensitized to birch and ragweed pollen, two airborne allergens that appeared only recently in the surroundings of Milan, were much older (in some cases >70 years old) than those developing allergies to 'old' airborne allergens, and that these subjects lacked any relevant predisposing factor, such as a family history of allergic diseases [15]. These observations suggested the existence of a specific susceptibility that persists until the 'appropriate' allergen is encountered. A subsequent follow-up study performed at this allergy center on patients living in the same area investigated the pattern of new sensitization among 726 patients monosensitized to different airborne allergens. After an average of 6–9 years of follow-up, the overall prevalence of new sensitization was 35% (256/726). Interestingly, 'new' allergens (birch and ragweed) were responsible for 89% of new sensitization [unpublished].

The present study retrospectively investigated de novo sensitization to birch and/or ragweed pollen, in a large number of monosensitized patients with different respiratory allergies who were routinely visited, followed up and, in several cases, received SIT at this allergy center during a 16-year period (January 1st, 1986, and December 31st, 2001). During the follow-up period patients receiving SIT became more frequently sensitized to the two allergens than subjects not receiving SIT. In the former, new sensitization appeared after the interruption of the 3-year injection SIT course in most cases, but about 10% of them became sensitized while undergoing injection immuno-

therapy. Ostensibly, this finding might lead one to conclude that SIT increased the risk of sensitization to these two allergens. However, the fact that allocation to SIT was on the basis of patient choice (i.e., the study was not randomized and placebo-controlled) probably influenced the results. In fact, although patients receiving and not receiving SIT did not show any baseline difference in age, sex or severity of allergic symptoms (as shown by the prevalence of asthma in the different subgroups) one has to consider that patients who are willing to undergo a 3-year injection SIT course might be more severely allergic than those refusing such therapy because they feel that their symptoms can be adequately controlled by anti-allergic drugs alone. Therefore, the different prevalence of new sensitization observed in the two groups of patients might simply reflect the fact that a more severe respiratory allergy to a single allergen is more frequently associated with a genetic predisposition to develop allergic responses to other airborne allergens as well. A higher prevalence of new sensitization in patients with both allergic rhinitis and asthma vs. patients with allergic rhinitis alone was also observed in another study [7] and supports this view. However, surprisingly enough, this did not seem to hold true for de novo sensitization to 'traditional' airborne allergens, that occurred with the same, low prevalence in patients receiving and not receiving SIT (4%). This finding suggests that the adult cohort studied here included very few subjects who were genetically predisposed to become sensitized to 'traditional' airborne allergens other than those causing primary sensitization; probably, those showing such predisposition had already become allergic at younger ages. In contrast, the study group included many subjects prone to become sensitized to the 'new' airborne allergens, birch and ragweed pollen; probably, when these subjects were enrolled, they had not yet been exposed to these 'new' allergens long enough to become sensitized. Whether this interpretation is correct or not, the results of this study suggest that, at least in adult patients, SIT does not exert any protective effect against sensitization to other classes of aeroallergens, a conclusion that is clearly in contrast with the findings of previous studies [5–7].

A striking difference between this study and the previous ones is represented by the prevalence of new sensitizations in the control groups. The overall rate of new sensitizations observed here (37%) was far lower than that recorded in those studies [5–7]. Such a marked discrepancy may be, at least in part, explained by some relevant differences in the design of the studies. Two studies [5, 6] were carried out on a pediatric population allergic to

house dust mite. Since sensitization to pollen allergens generally occurs at older ages than sensitization to house dust mite [16, 17], it cannot be excluded that many of the children in those studies carried a genetic predisposition to pollen allergy that was still clinically unexpressed when they were enrolled. An increased sensitization rate from childhood to adulthood has been shown by several longitudinal studies [18–20], and this propensity seems particularly evident in children sensitized to house dust mites [20]. In the present study, patients allergic to house dust mite, whether or not they received SIT, showed a lower propensity to become sensitized to birch and/or ragweed pollen suggesting that adults monosensitized to house dust mite might represent a subset of allergic patients who are genetically not prone to become sensitized to other airborne allergens as well. The third study was performed in adults [7]; in that study 68% and 78% control subjects had become multisensitized after 4 and 7 years of follow-up, respectively (in the SIT group these proportions were 24 and 27%, respectively). If those results really mirrored the events in the general population of allergic patients, nearly all subjects not receiving SIT would be expected to become multisensitized, which is certainly not the case in daily practice. Probably, some peculiarity in admission criteria (e.g., the enrollment of subjects showing slight skin reactivity to one or more allergens other than the one causing primary sensitization) is the only reasonable explanation for that impressive rate of new sensitizations.

The clinical success of SIT is thought to rely essentially on two main immunological mechanisms: (a) Immune deviation, a shift in the balance of Th1/Th2 responses in favor of the former one, possibly mediated by IL-12, the strongest inducer of Th1 responses known [21, 22]; and

(b) Immune tolerance, induced by the immunosuppressive cytokine IL-10 [23, 24]. Together, these events lead to a reduction in the production of cytokines by allergen-specific Th2 cells [25], which profoundly influences the production of specific IgE, priming and recruitment of mast cells, activation of eosinophils, and lymphoproliferative responses [26–28]. All these effects are specific, as responses to control allergens are unchanged [23, 29]. Recently, Moverare et al. [30] observed novel sensitization to cross-reacting allergens contained in birch pollen extract, namely Bet v 2 (birch profilin) and Bet v 4 (a calcium-binding allergen), in a significant proportion of patients submitted to SIT with birch pollen extract. At least some observations seem to exclude that novel sensitizations in this study were caused by cross-reactive allergens contained in pollen extracts. First, novel sensitization to birch and/or ragweed pollen occurred both in patients and controls. Second, and more important, patients developing new sensitization did not show any skin reactivity to pollen extracts other than those involved in new sensitization, thus suggesting that such sensitizations were not caused by cross-reacting allergens. With the possible exception of some cross-reacting allergens [30] there is no immunological evidence that allergen SIT may influence the immune response (i.e. prevent sensitization) to allergens other than the relevant one. Genetic predisposition of the individual towards developing a Th2 response to specific allergens is one of the key determinants of allergenicity. This study suggests that in adult, monosensitized patients SIT is not able to influence predisposition to become allergic to other airborne allergies (both 'new' and 'traditional'), and therefore does not exert any protective effect in this sense.

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