Pollen Specific Immunotherapy Is Not a Risk Factor for De Novo Sensitization to Cross-Reacting Allergens in Monosensitized Subjects

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Abstract. Background: Some studies have suggested that specific immunotherapy (SIT) may cause de novo sensitization to allergenic proteins to which patients were not previously allergic. This event might theoretically involve cross-reacting pollen allergens, such as profilin or polcalcins, posing a risk of SIT-induced polysensitization to pollens in patients who were originally monosensitized.

Objectives: The aim of this study was to assess whether injection SIT with commercial pollen extract represents a risk factor for the de novo development of sensitization to different pollens in monosensitized patients.

Methods: The study involved 142 subjects diagnosed as being monosensitized to a single pollen: 64 patients who were administered a 3-year course of injection SIT and 78 controls. Subjects underwent control skin prick tests (SPT) with a series of 8 seasonal airborne allergens at least 3 years after the first visit. Patients with 5 or more new sensitivities on SPT were considered to be de novo polysensitized.

Results: At the end of the 3-year follow-up period, the proportion of polysensitized subjects was identical in previously monosensitized patients who underwent SIT and control individuals (11% and 10%, respectively). Individuals who were polysensitized were significantly younger than those who were not (mean age±SD, 21.6±11.0 years vs 31.6±15.6 years; P < .05).

Conclusion: SIT does not represent a risk factor for progression towards multiple pollen sensitization in monosensitized pollen-allergic patients.

Key words: Pollen. Specific immunotherapy. Calcium binding proteins. Profilin. Cross-reactivity.

Resumen. Antecedentes: Algunos estudios han sugerido que la inmunoterapia específica (ITE) puede causar sensibilización de novo a proteínas alérgicas a las que los pacientes no eran alérgicos anteriormente. Este hecho podría implicar, teóricamente, una reacción cruzada de alérgenos del polen, como la profilina o polcalcinas, provocando un riesgo de polisensibilización a polenes por la ITE en pacientes inicialmente monosensibilizados.

Objetivos: El propósito del estudio fue valorar si la inoculación de la ITE con extracto de polen comercial representa un factor de riesgo para el desarrollo de novo de sensibilización a distintos polenes en pacientes monosensibilizados.

Métodos: Participaron en el estudio 142 sujetos diagnosticados como monosensibilizados a un único polen: 64 pacientes a los que se administró una tanda de inyecciones de ITE de 3 años de duración y 78 controles. Los sujetos se sometieron a pruebas cutáneas de control con una serie de 8 alérgenos inhalantes estacionales durante 3 años como mínimo después de la primera visita. Los pacientes con 5 sensibilizaciones nuevas o más en las pruebas cutáneas se consideraron polisensibilizados de novo.

Resultados: Al finalizar el periodo de seguimiento de 3 años de duración, la proporción de sujetos polisensibilizados fue idéntica entre los pacientes previamente monosensibilizados que se sometieron a la ITE y los controles (11% y 10%, respectivamente). Los sujetos polisensibilizados eran significativamente más jóvenes que los que no presentaron esta característica (edad media±DE, 21.6±11.0 años frente a 31.6±15.6 años; P < .05).

Conclusión: La ITE no representa un factor de riesgo para desarrollar una sensibilización a diversos polenes en pacientes alérgicos al polen monosensibilizados.

Introduction

Allergen-specific immunotherapy (SIT) is an established method for the treatment of respiratory allergy and is the only antigen-specific immunomodulatory treatment presently available. Its efficacy in allergic rhinoconjunctivitis and asthma has been thoroughly demonstrated by a large number of properly performed studies [1-5]. As commercial extracts used to carry out SIT are obtained from whole allergenic sources, they contain a mixture of both allergenic and nonallergenic proteins, and the former include allergens that are relevant for some patients as well as allergens to which patients are not sensitized. This carries a theoretical risk of de novo, SIT-induced sensitization.

The possible induction of new sensitizations through SIT has received comparatively little attention. Some studies have reported the appearance of IgE specific for new allergenic components of extracts used for SIT [6-12] and other researchers have observed that SIT with house dust mites may cause the appearance of new cross-reacting IgE antibodies to snail and shrimp and a worsening of clinical symptoms after the ingestion of these foods [13,14]. Ball et al [7] found an IgE and IgG4 response to Phl p1 epitopes not recognized by the patients before SIT, and in 1 case they observed the de novo appearance of IgE against a new grass pollen allergen. Moverare et al [6] showed the induction of new IgE specificities to individual birch pollen allergens in 65% of birch-pollen allergic patients submitted to rush immunotherapy; the combined incidence of new IgE specificities to either birch profilin, Bet v 2, or birch calcium binding protein, Bet v 4, was 29%. Similarly, Modrzynski et al [12] detected the appearance of IgE specific for Bet v 2 in 5 out of 12 patients originally monosensitized to Bet v 1 after they started SIT with birch pollen extract. The clinical relevance of those findings remains elusive.

Pollen allergens include some highly cross-reactive proteins. Profilin, the so-called pan-allergen, is widely distributed throughout the plant kingdom and patients sensitized to it may have clinical symptoms following exposure to a number of distinct pollens, as well as to vegetable foodstuffs [15-23]. The calcium binding proteins polcalcins are not present in vegetable foods but have been detected in all pollens studied so far and are clinically relevant [24, 25]. Clearly, an SIT-induced de novo sensitization to these cross-reacting allergens would be particularly worrying as corecognition of the same allergen in different sources might cause clinical polysensitization. The aim of the present study was to detect whether and how frequently pollen SIT is associated with the development of multiple pollen skin reactivity suggestive of de novo sensitization to cross-reacting pollen allergens and to investigate the clinical relevance of these phenomena.

Methods

Patients

The study included 142 subjects: 70 men and 72 women; mean age, 30.6 years (range, 8-70 years). All subjects attended the Allergy Department of the Ospedale Caduti Bollatesi, Bollate (MI), Italy between January 1998 and June 2001. All had been diagnosed as being monosensitized to a single pollen. Monosensitization was defined as hypersensitivity to only 1 of 8 pollen extracts (see below), in keeping with clinical symptoms (seasonal rhinoconjunctivitis with or without asthma). All subjects were offered injection SIT. The 64 patients who accepted (SIT patients) underwent a 3-year course of injection SIT that was completed in all cases by the end of March, 2005; the remaining 78 subjects who declined SIT (because of lack of time due to work problems, fear of adverse reactions, fear of injections, etc) served as controls. SIT patients and control patients had a similar mean age (31.5 [range, 8-68] years and 30.0 [range, 8-70] years, respectively; \( P \) not significant [NS]) and sex distribution (ratio of men to women, 31/33 vs 39/39; \( P = \) NS). Furthermore, the 2 groups did not show any significant difference in sensitization to individual pollens, prevalence of asthma, symptom severity at the beginning of the study (as evaluated by symptom scores and use of rescue therapy), or family history of allergic diseases.

Table 1. Clinical Features of Patients and Controls*

<table>
<thead>
<tr>
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<th>Patients (n=64)</th>
<th>Controls (n=78)</th>
<th>( P )</th>
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</thead>
<tbody>
<tr>
<td>Mean age (range), y</td>
<td>31.5 (8-68)</td>
<td>30.0 (8-70)</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>31/33</td>
<td>39/39</td>
<td>NS</td>
</tr>
<tr>
<td>Primary sensitization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grass</td>
<td>37 (58%)</td>
<td>48 (62%)</td>
<td>NS</td>
</tr>
<tr>
<td>Pellitory</td>
<td>7 (11%)</td>
<td>2 (3%)</td>
<td>NS</td>
</tr>
<tr>
<td>Mugwort</td>
<td>2 (3%)</td>
<td>0 (0%)</td>
<td>NS</td>
</tr>
<tr>
<td>Ragweed</td>
<td>6 (9%)</td>
<td>15 (19%)</td>
<td>NS</td>
</tr>
<tr>
<td>Birch</td>
<td>12 (19%)</td>
<td>12 (15%)</td>
<td>NS</td>
</tr>
<tr>
<td>Cypressus</td>
<td>0 (0%)</td>
<td>1 (1%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Data are shown as number (%) unless otherwise indicated. NS indicates not significant; M, male; F, female.
main clinical features of patients and controls are compared in Table 1.

At the end of the 3-year course of SIT (SIT patients) or at least 3 years after the first visit (control patients) all participants underwent a follow-up evaluation including a thorough interview about the possible onset of respiratory symptoms in different seasons and skin prick tests (SPT) with the whole panel of 8 seasonal airborne allergens. Novel sensitization to cross-reacting pollen allergens was diagnosed based on the presence of skin reactivity to at least 6 pollens, including the original one, with or without clinical symptoms [25]. Sensitization to 5 rather than all 7 additional pollen extracts was considered sufficient to diagnose production of IgE to cross-reacting pollen allergens because *Parietaria* profilin shows limited and variable cross-reactivity with profilins from other pollens [26], and this also seems to be the case for cypress profilin (RA, unpublished data).

**Skin Tests**

Both at the first visit and at the 3-year follow-up evaluation, patients and controls underwent SPT with commercial pollen extracts (Allergopharma, Reinbeck, Germany; 50 000 standardized biological units/mL) of 8 seasonal airborne allergen sources, including grasses, weeds (mugwort, ragweed, pellitory, and plantain) and trees (birch, olive, and cypress). SPT were carried out and assessed following established methods [27]; wheals showing a mean diameter of more than 3 mm were considered positive. Histamine (10 mg/mL) and saline were used as positive and negative controls, respectively. All subjects had stopped treatment with antihistamines at least 4 days before SPT were performed.

**Immunotherapy**

SIT patients underwent injection immunotherapy with commercial depot aluminum hydroxide-adsorbed pollen extracts (Allergopharma, Reinbeck, Germany). Weekly doses were given during the “build-up” (induction) phase in order to reach the planned maintenance dose (1.0 mL of the final vial). However, this was not achieved in all patients due to adverse reactions. Subjects were therefore maintained on the highest tolerated dose that did not elicit side effects. It has been proposed that this corresponds to the optimal dose [1]. Maintenance doses were given at 3- to 4-week intervals in all patients. Maintenance doses were reduced by half during the specific pollen season. All injections were performed by the author at the Allergy Unit of Ospedale Caduti Bollatesi and all patients completed the 3-year course of SIT.

**Statistical Analysis**

Means were compared using the 2-tailed Student t test. Proportions were compared with the $\chi^2$ test with Yate’s correction. $P$ values of less than .05 were considered statistically significant.

**Results**

At the end of the 3-year follow-up period a total of 15 subjects showed polysensitization (ie, skin reactivity to at least 5 pollen extracts other than the original sensitizing extract) and a further 26 subjects had become sensitized to 1 additional pollen other than the original. The proportion of subjects who had become polysensitized

<table>
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<th>Table 2. New Sensitizations at the End of the Follow-Up Period *</th>
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<tr>
<td>Number sensitized to 1 new pollen at follow-up visit</td>
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<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Number polysensitized at follow-up visit</td>
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<tr>
<td>Number polysensitized at follow-up visit</td>
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* Data are shown as number (%). Polysensitization refers to positive skin prick test to 6 out of 8 pollen extracts. NS indicates not significant.

<table>
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<th>Table 3. Analysis of Novel Sensitizations According to Primary Sensitization*</th>
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<td>Primary Sensitization</td>
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<tr>
<td>------------------------</td>
</tr>
<tr>
<td>G (n = 37)</td>
</tr>
<tr>
<td>P (n = 7)</td>
</tr>
<tr>
<td>M (n = 2)</td>
</tr>
<tr>
<td>R (n = 6)</td>
</tr>
<tr>
<td>B (n = 12)</td>
</tr>
<tr>
<td>C (n = 0)</td>
</tr>
</tbody>
</table>

*Data are shown as number of patients. R indicates ragweed; B, birch; G, grass; C, cypress; P, pellitory; M, mugwort; Poly, polysensitization.
was identical in SIT patients and controls (11% and 10%, respectively; \( P = \text{NS} \); Table 2). Subjects who became polysensitized were significantly younger than those who did not develop polysensitization (mean age, 21.6 ± 11.0 vs 31.6 ± 15.6 years; \( P < .05 \)); this was observed in both patient and control groups. De novo sensitization to 1 pollen other than the original was only slightly more frequent among control patients (21% vs 16%; \( P = \text{NS} \)). Most of the novel single sensitizations that occurred (10 SIT patients and 16 controls) were caused by ragweed (n = 16) or birch (n = 7); grass, pellitory, and cypress only caused new sensitizations in isolated cases. Novel sensitization to either a single source or several allergen sources was not associated with any specific baseline allergenic source (Table 3).

**Discussion**

While novel, SIT-induced sensitization to different proteins belonging to the primary sensitizing allergen source should have limited clinical impact, the onset of IgE reactivity to cross-reacting pollen allergens, such as profilin and calcium binding protein, might theoretically result in a widening of the symptomatic period from January (start of the cypress pollen season) to October (end of the ragweed and mugwort pollen season). The present work focused on this latter aspect, using skin reactivity to at least 6 out of 8 commercial pollen extracts as a clinical marker of sensitization to cross-reacting pollen allergens. The presence of cross-reacting allergens in the extracts used for this study has been demonstrated previously. In 2 studies of profilin hypersensitivity, patients with circulating IgE to rBet v 2 and *Phleum* profilin were all positive on SPT with grass, mugwort, ragweed, plantain, birch, hazel, olive, and cypress pollen extracts (produced by Allergopharma); in contrast, no patient sensitized to only 1 pollen showed IgE reactivity to profilin [23,26]. Similarly, Mari [24] found that hypersensitivity to the pan-allergens profilin and calcium binding protein was associated with multiple skin reactivity to pollen extracts on SPT. Another study showed the disappearance of fennel, cucumber, and melon allergy in a patient submitted to injection SIT with grass, ragweed and mugwort extracts from the same producer, suggesting the presence of profilin in those extracts [28]. Finally, the presence of calcium binding protein in *Phleum* extract has been directly detected at Allergopharma laboratories using a Phl p 7-specific monoclonal antibody (Dr Oliver Cromwell, e-mail communication, 2005). Thus, it is reasonable to assume that these extracts for SPT show a high positive and negative predictive value in the detection of sensitization to profilin or calcium binding protein, and that patients undergoing injection SIT are exposed to cross-reacting pollen allergens.

Based on the results of the present study, pollen SIT does not seem to represent a risk factor for the development of hypersensitivity to cross-reacting allergens. This phenomenon occurred in a limited, and nearly identical, proportion of both SIT patients and control patients (about 10%). These findings are in keeping with the results of another recent prospective study [29]. The rather frequent de novo sensitization to ragweed and birch pollen detected both in patients and controls confirms previous observations from this geographical area, where these allergen sources are relatively “new” and show a marked tendency to sensitize patients of all ages [30, 31]. Patients who developed pollen polysensitization were significantly younger than those who did not. This observation is consistent with some previous studies showing that new sensitizations to airborne allergens are particularly frequent in younger patients [32, 33].

Genetic predisposition of the individual towards developing a T helper 2 response to specific allergens is a key determinant of allergenicity. Although some studies suggest that the level of allergen exposure is a relevant factor in sensitization [34-36], the present work, carried out mostly with adults, suggests that sensitization as a result of the repeated administration of a specific allergen is an infrequent event in subjects who are not prone to become allergic to that particular protein.

**References**


