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

R Asero

Ambulatorio di Allergologia, Clinica San Carlo, Paderno Dugnano (MI), Italy

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 $\pm$ 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 alergénicas 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 pólenes 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 pólenes 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 período 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 $\pm$ DE, $21,6 \pm 11,0$ años frente a $31,6 \pm 15,6$ años; $P < 0,05$).

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

Palabras clave: Polen. Inmunoterapia específica. Proteínas fijadoras de calcio. Profilina. Reactividad cruzada.

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 p 1 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. The

Table 1. Clinical Features of Patients and Controls*

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

*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; 50000 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 $\chi2$ test with Yates' 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 2. New Sensitizations at the End of the Follow-Up Period *

	Patients	Controls	*P*
Number sensitized to 1 new pollen at follow-up visit	10 (16%)	16 (21%)	NS
Number polysensitized at follow-up visit	7 (11%)	8 (10%)	NS

* Data are shown as number (%). Polysensitization refers to positive skin prick test to 6 out of 8 pollen extracts. NS indicates not significant.

Table 3. Analysis of Novel Sensitizations According to Primary Sensitization*

Patients		Controls	
Primary Sensitization	Novel Sensitizations	Primary Sensitization	Novel Sensitizations
G (n = 37)	3 R; 4 B; 5 Poly	G (n = 48)	6 R; 1 P; 1C; 3 B; 7 Poly
P (n = 7)	1 R	P (n = 2)	–
M (n = 2)	–	M (n = 0)	–
R (n = 6)	1Poly	R (n = 15)	–
B (n = 12)	2 R; 1 Poly	B (n = 12)	1 G; 3 R
C (n = 0)	–	C (n = 1)	1 R

*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 = 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 = 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

1. Malling HJ, Weeke B, eds. EAACI Position paper: Immunotherapy. Allergy. 1993; 48 Suppl 14:S9-34.
2. Bousquet J, Lockey RF, Malling HJ. WHO position paper. Allergen immunotherapy: therapeutic vaccines for allergic disease. Allergy. 1998; 53 Suppl 44:S1-42.
3. Jacobsen L, Nuchel-Petersen B, Wihl JA, Lowenstein H, Ipsen H. Immunotherapy with partially purified and standardized tree pollen extract. IV. Results from long-term (6-year) follow-up. Allergy. 1997; 52:914-20.
4. Durham SR, Walker SM, Varga EM, Jacobson MR, O'Brien F, Noble W, Till SJ, Hamid QA, Nouri Aria KT. Long-term clinical efficacy of grass-pollen immunotherapy. N Engl J Med. 1999; 341:468-75.
5. Bousquet J, Hejjaoui A, Clauzel AM, Guerin B, Duivert H, Skassa-Brociek W, Miche FB. Specific immunotherapy with a standardized Dermatophagoides pteronyssinus extract. II. Prediction of efficacy of immunotherapy. J Allergy Clin Immunol. 1988; 82:971-7.
6. Moverare R, Elfman L, Vesterinen E, Metso T, Haathela T. Development of new IgE specificities to allergenic components in birch pollen extract during specific immunotherapy studied with immunoblotting and Pharmacia CAP system. Allergy. 2002; 57:423-30.
7. Ball T, Sperr WR, Valent P, Lidholm J, Spitzauer S, Ebner C, Kraft D, Valenta R. Induction of antibody responses to new B cell epitopes indicates vaccination character of allergen immunotherapy. Eur J Immunol. 1999; 29:2026-36.
8. Moller C, Dreborg S, Einarsson R. Immunotherapy to deciduous tree pollens: specific IgE and IgG antibody patterns. Clin Allergy. 1987; 17:551-62.
9. Richter M, Sehon AH, Gordon J, Gregoire C, Rose B. Demonstration of regains to a new specificity in sera of treated ragweed sensitive persons. J Allergy. 1958; 29:287-92.

10. Birkner T, Rumpold H, Jarolim E, Ebner H, Breitenbach M, Skvaric F, Scheiner O, Kraft D. Evaluation of immunotherapy-induced changes in specific IgE, IgG and IgG subclasses in birch pollen allergic patients by means of immunoblotting. Correlation with clinical response. Allergy. 1990; 45:418-26.

11. Juarez C, Blanca M, Miranda A, Sanchez F, Carmona MJ, Avila MJ, Fernandez S, Fernandez J, Terrados S. Specific IgE antibodies to vespids in the course of immunotherapy with Vespula germanica administered to patients sensitized to Polistes dominulus. Allergy. 1992; 47:299-302.

12. Modrzynski M, Zawisza E. Possible induction of oral allergy syndrome during specific immunotherapy in patients sensitive to tree pollen. Med Sci Monit. 2005; 11:CR351-5.

13. Van Ree R, Antonicelli L, Akkerdaas JH, Garritani MS, Aalberse RC, Bonifazi F. Possible induction of food allergy during mite immunotherapy. Allergy. 1996; 51:108-13.

14. Pajno GB, La Grutta S, Barberio G, Canonica GW, Passalacqua G. Harmful effect of immunotherapy in children with combined snail and mite allergy. J Allergy Clin Immunol. 2002; 109:627-9.

15. Ebner C, Hirschwehr R, Bauer L, Breiteneder H, Valenta R, Ebner H, Kraft D, Scheiner O. Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). J Allergy Clin Immunol. 1995; 95:962-9.

16. van Ree R, Fernandez-Rivas M, Cuevas M, van Wijngaarden M, Alberse RC. Pollen-related allergy to peach and apple: an important role for profilin. J Allergy Clin Immunol. 1995; 95:726-34.

17. Petersen A, Vieths S, Aulepp H, Schlaak M, Becker WM. Ubiquitous structures responsible for cross-reactivity between tomato fruit and grass pollen allergens. J Allergy Clin Immunol. 1996; 98:805-15.

18. Fritsch R, Ebner H, Kraft D, Ebner C. Food allergy to pumpkin seed; characterization of the allergens. Allergy. 1997; 52:335-7.

19. Reindl J, Anliker MD, Karamloo F, Vieths S, Wuthrich B. Allergy caused by ingestion of zucchini (Cucurbita pepo): characterization of allergens and cross-reactivity to pollen and other foods. J Allergy Clin Immunol. 2000; 106:379-85.

20. Fah J, Wuthrich B, Vieths S. Anaphylactic reaction to lychee fruit: evidence for sensitization to profilin. Clin Exp Allergy. 1995; 25:1018-23.

21. Reindl J, Rihs HP, Scheurer S, Wangorsch A, Haustein D, Vieths S. IgE reactivity to profilin in pollen-sensitized subjects with adverse reactions to banana and pineapple. Int Arch Allergy Immunol. 2002; 128:105-14.

22. Anliker MD, Reindl J, Vieths S, Wuthrich B. Allergy caused by the ingestion of persimmon (Diospyros kaki): detection of specific IgE and cross-reactivity to profilin and carbohydrate determinants. J Allergy Clin Immunol. 2001; 107:718-23.

23. Asero R, Mistrello G, Roncarolo D, Amato S, Zanoni D, Barocci F, Caldironi G. Detection of clinical markers of sensitization to profilin in patients allergic to plant-derived foods. J Allergy Clin Immunol. 2003; 112:427-32.

24. Mari A. Multiple pollen sensitization: a molecular approach to the diagnosis. Int Arch Allergy Immunol. 2001; 125:57-65.

25. Ferreira F, Hawranek T, Gruber P, Wopfner N, Mari A. Allergic cross-reactivity: from gene to the clinic. Allergy. 2004; 59:243-67.

26. Asero R, Mistrello G, Roncarolo D, Amato S. Parietaria profilin shows only limited cross-reactivity with birch and grass profilins. Int Arch Allergy Immunol. 2004; 133:121-4.

27. Dreborg S, Frew A. Allergen standardization and skin tests. EAACI position paper. Allergy. 1993; 48:49-75.

28. Asero R. Fennel, cucumber, and melon allergy successfully treated with pollen-specific injection immunotherapy. Ann Allergy Asthma Immunol. 2000; 84:460-2.

29. Tella R, Bartra J, San Miguel M, Olona M, Bosque M, Gaig P, Garcia-Ortega P. Effects of specific immunotherapy on the development of new sensitizations in monosensitized patients. Allergol Immunopathol. 2003; 31:221-5.

30. Asero R. Birch and ragweed pollinosis north of Milan: a model to investigate the effects of exposure to "new" airborne allergens. Allergy. 2002; 57:1063-6.

31. Asero R. Analysis of new respiratory allergies in patients monosensitized to airborne allergens in the area north of Milan. J Investig Allergol Clin Imunol. 2004; 14:208-13.

32. Silvestri M, Rossi GA, Cozzani S, Pulvirenti G, Fasce L. Age-dependent tendency to become sensitized to other classes of aeroallergens in atopic asthmatic children. Ann Allergy Asthma Immunol. 1999; 83:335-40.

33. Fasce L, Tosca MA, Olcese R, Milanese M, Erba D, Ciprandi G. The natural history of allergy: the development of new sensitizations in asthmatic children. Immunol Lett. 2004; 93:45-50.

34. Lau S, Falkenhorst G, Weber A, Werthmann I, Lind P, Buettner-Goetz P, Wahn U. High mite-allergen exposure increases the risk of sensitization in atopic children and young adults. J Allergy Clin Immunol. 1989; 84:718-26.

35. Kuehr J, Frischer T, Meinert R, Barth R, Forster J, Schraub S, Urbanek R, Karmaus W. Mite allergen exposure is a risk for the incidence of sensitization. J Allergy Clin Immunol. 1994; 94:44-52.

36. Wahn U, Lau S, Bergmann R, Kulig M, Forster J, Bergmann K, Bauer CP, Guggenmoos-Holzmann I. Indoor allergen exposure is a risk factor for sensitization during the first three years of life. J Allergy Clin Immunol. 1997; 99:763-9.

Riccardo Asero

Ambulatorio di Allergologia
Clinica San Carlo
Via Ospedale 21
20037 Paderno Dugnano (MI), Italy
E-mail: r.asero@libero.it