

Immunotherapy Is Allergen-Specific: A Double-Blind Trial of Mite or Timothy Extract in Mite and Grass Dual-Allergic Patients

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Key Words

Immunotherapy • Conjunctival provocation test • Allergen-specific IgG • Double-blind trial • Placebo

Abstract

Background: One hundred years ago, Noon [Lancet 1911;1:1572–1573], using conjunctival provocation testing (CPT), was the first to demonstrate the effectiveness of subcutaneous immunotherapy (SCIT) in grass-allergic subjects with hay fever. In this centenary year, we present data that, by use of CPT and allergen-specific IgG, replicate this observation and additionally confirm the allergen specificity of SCIT by using a double-blind design employing *either* grass or mite SCIT in dual grass- and mite-allergic individuals. **Methods:** Twenty adults (11 females) with perennial rhinoconjunctivitis and exacerbation of symptoms during the grass pollen season and in the autumn had immediate skin and conjunctival sensitivity and raised specific IgE to both *Dermatophagoides farinae* and *Phleum pratense*. Participants were randomly assigned to either timothy or *D. farinae* immunotherapy for 3 years. CPT and specific IgG tests to both allergens were performed annually. After 3 years, subjects gave their blinded overall evaluation. **Results:** Six mild-to-moderate general reactions occurred in 2 timothy- and 4 mite-treated patients. Four of these patients and 2 other patients withdrew from the study. Seven patients in each

group completed the study. After 3 years of immunotherapy, the timothy CPT threshold concentration had increased 16-fold in timothy-treated patients ($p < 0.05$; between-group change, $p < 0.05$). The increase in the mite CPT threshold in mite- compared to grass-treated patients was 31-fold ($p < 0.05$). The overall assessment of conjunctival sensitivity was highly significant in favour of treatment ($p < 0.015$), as was that of allergen-specific IgG ($p < 0.0001$). **Conclusions:** Allergen immunotherapy is allergen species-specific, as judged by decreased conjunctival sensitivity and changes in allergen-specific IgG concentrations.

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Introduction

It is 100 years since Noon [1] first demonstrated the efficacy of subcutaneous immunotherapy (SCIT). SCIT with allergen extracts/vaccines has been named specific immunotherapy, allergen-specific immunotherapy and allergen vaccination. Thus, in the World Health Organisation position paper on allergen immunotherapy [2], it was stated: 'The response to immunotherapy is specific for the antigen administered.' In that paper as well as in

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Table 1. Patient characteristics before starting SCIT

	Immunotherapy treatment groups	
	timothy	<i>D. farinae</i>
Patients included, n	9	11
Drop-outs, n	2	4
Patients left after 3 years, n	7	7
Seasonal symptoms, n		
Grass season	9	11
Worsening in the autumn	9	11
SPT wheal, mm ²		
Timothy	17 (8–38)	26 (11–48)
<i>D. farinae</i>	52 (18–119)	24 (9–76)
CPT threshold concentration, BU/ml		
Timothy	4,541 (316–100,000)	2,591 (316–100,000)
<i>D. farinae</i>	615 (100–10,000)	643 (100–3,162)
Allergen-specific IgE, PRU/ml		
Timothy	1.76 (0.40–7.65)	5.84 (1.84–18.5)
<i>D. farinae</i>	4.34 (0.98–19.3)	1.48 (0.14–16.1)
Allergen-specific IgG, U/ml		
Timothy	12.9 (<10–166)	7.1 (<10–56)
<i>D. farinae</i>	23.3 (<10–179)	37.9 (<10–78.9)

Except where indicated otherwise, values represent geometric means with ranges in parentheses. PRU = Phadebas RAST units.

many other papers, the specificity of SCIT has not been questioned, with one notable exception [3]. Thus, in a randomised double-blind placebo-controlled trial of subcutaneous ragweed immunotherapy in adults with dual sensitivity to ragweed and grass pollen, seasonal symptom scores decreased during the ragweed but not during the grass pollen season [3]. In an open study in 10 children with dual sensitivity to mite and cat, subcutaneous house dust mite immunotherapy resulted in a significant reduction in skin and nasal reactivity and immunological responses to mite, but not to cat allergen [4].

In contrast, a recent trial of open sublingual pollen immunotherapy in dual birch- and grass-sensitized patients questioned the allergen specificity, although the lack of a difference in symptom scores during the grass and birch seasons for participants treated with either allergen could have been explained by a placebo effect alone, and no placebo group was included to test this [5].

To our knowledge, there has been only one study of SCIT that used dual birch- and grass-sensitive patients [6] and blinded administration of one or the other allergen in order to test the allergen specificity of immunotherapy. Unfortunately, this study in adults was hampered by the fact that pollen counts during the grass season were too

low to allow a convincing evaluation of allergen specificity using symptom scores.

In the beginning of the 1980s, when this trial was performed, we had the experience when testing histamine placebo that subcutaneous injection of histamine caused a different sensation than allergen injections. Thus, we designed a study to test the efficacy of mite (*Dermatophagoides farinae*) and grass (*Phleum pratense*, timothy) SCIT in patients clinically sensitive to both allergens, in a double-blinded manner, with the other allergen used as placebo, without using histamine placebo, and in addition, to evaluate the allergen specificity of SCIT.

Patients and Methods

Patients

Twenty adults, 17–55 years of age (mean 29 years; 11 females and 9 males), with a history of perennial rhinoconjunctivitis without clinical asthma with seasonal symptoms during the grass pollen season (table 1) were included in the study. All had positive skin prick tests (SPTs) with *D. farinae* and timothy pollen allergen extracts [100,000 Nordic Biological Units (BU)/ml] [7, 8]. Furthermore, all patients had a positive conjunctival provocation test (CPT) with both *D. farinae* and timothy (100,000 BU/ml or less),

and all had anti-*D. farinae* and anti-timothy serum (s)-IgE ≥ 0.35 Phadebas RAST units/ml (Phadebas RAST®, Pharmacia Diagnostics, Uppsala, Sweden). None of the subjects had received immunotherapy with mite or grass pollen allergen extract in the last 5 years and none had received systemic corticosteroids within the previous 6 months, was pregnant or had any disease contraindicating allergen immunotherapy. Furthermore, no subject had a history of symptoms upon exposure to cats, dogs or other pet animals. The study was approved by the Royal Brompton Hospital ethics committee and performed with the informed written consent of the participants.

Design of the Study

Patients were randomly assigned to mite ($n = 11$) or timothy ($n = 9$) SCIT that was given in a partly clustered regimen with a dose increase every second week. Blood sampling for IgG measurements and CPTs was performed at baseline and then yearly for 3 years.

Allergen Extracts/Vaccines

Biologically standardized, partly purified *D. farinae* and timothy pollen allergen extracts, freeze-dried in vials in 4 concentrations (100, 1,000, 10,000 and 100,000 BU/ml; Pharmalgen®, Pharmacia Diagnostics AB) were used for both in vivo diagnosis and SCIT. Pharmalgen timothy and *D. farinae* freeze-dried extracts (100,000 BU/ml) contained approximately 100 µg of major allergen [9]. For immunotherapy injections during the up dosing phase (up to about 40,000 BU) and for CPT and SPT, the extracts were reconstituted with Albumin diluent® (0.03% human serum albumin in phosphate buffer, Pharmacia Diagnostics). For up dosing from 40,000 to about 100,000 BU and for maintenance immunotherapy, extracts were diluted with Depot diluent® [0.3% Al(OH)₃, Pharmacia Diagnostics]. For SPT and CPT, Albumin diluent was used as negative control.

Conjunctival Provocation Tests

The CPTs were performed with the freeze-dried timothy or *D. farinae* extract reconstituted with Albumin diluent, starting with 1 drop of diluent instilled in one eye, and then, after 10 min, provided there was no reaction, 10 BU/ml (approx. 10 ng/ml major allergen) was instilled in the other conjunctival sac. The dose was increased, by half ¹⁰log steps, every 10 min using alternate eyes to a maximum of 1,000,000 BU/ml (approx. 1 mg of major allergen/ml) [9]. The threshold concentration was defined as the concentration that elicited itching of the eye and reddening of at least 50% of the conjunctiva sclera [10, 11].

Determination of *D. farinae*- and Timothy-Specific IgG Antibodies

Blood was drawn before the study and after 1, 2 and 3 years of immunotherapy. The samples were stored at -20°C until assayed.

Timothy- and *D. farinae*-specific s-IgG antibodies were determined using allergen-coated tubes, patient serum and rabbit anti-human IgG antibodies. The results were related to reference curves obtained using sera with high titres of timothy and *D. farinae* IgG antibodies, respectively, and expressed in arbitrary units (U) per millilitre.

Overall Assessment

After 3 years of SCIT, patients were requested to respond to a questionnaire grading their symptoms during the grass and mite

Table 2. The dose schedule during up dosing

Visit No.	Week	Dose BU	Dose ¹ µg	Total dose ¹ µg/day	Diluent
1	0	10	0.01	0.08	Albumin
		20	0.02		Albumin
		50	0.05		Albumin
2	2	100	0.1	0.8	Albumin
		200	0.2		Albumin
		500	0.5		Albumin
3	4	1,000	1 ²	1	Albumin
4	6	5,000	5 ²	5	Albumin
5	8	10,000	10 ²	10	Albumin
6	10	20,000	20 ²	20	Albumin
7	12	40,000	40 ²	40	Depot
8	14	60,000	60 ²	60	Depot
9	16	80,000	80 ²	80	Depot
10	18	100,000	100 ²	100	Depot

¹ These are approximate values.

² These doses could be divided into two depending on the size of the immediate skin response to the preceding dose.

seasons, respectively, compared to their symptoms before treatment. The scoring was graded as follows: much better = 1; better = 2; the same = 3; worse = 4, and much worse = 5.

Subcutaneous Immunotherapy

This study was performed in the early 1980s, before World Health Organisation guidelines [2] on dose recommendations were available (1998). At this time, immunotherapy regimens aimed at a monthly maintenance dose of 100,000 BU, i.e. approximately 100 µg of major allergen, which is 5–16 times higher than that currently recommended for SCIT with inhalant allergens. There was an ongoing discussion regarding whether *Dermaphagoides pteronyssinus* or *D. farinae* was most relevant for immunotherapy in mite-allergic patients, although considerable cross-reactivity was already known to exist. Since only *D. farinae* was available as a standardized extract according to version 1 of the Nordic Guidelines, this was chosen as a standardized extract of comparable quality to the timothy grass extract used. After patients were randomly allocated to treatment with either *D. farinae* or timothy allergen extract, SCIT was started with 10 BU (about 10 ng of major allergen) according to a clustered regimen, using the freeze-dried extract reconstituted with Albumin diluent (table 2). During the clustered regimen period, extracts were given every half an hour until a large local or general reaction occurred. When a dose of 40,000 BU was reached, patients were switched to extracts reconstituted with Depot diluent. When the highest tolerated dose was reached, maintenance therapy was given with the extracts reconstituted with an alum-based Depot diluent, firstly twice with 2-week intervals, then monthly for 3 years. Local and systemic side effects were recorded at/after each visit.

Table 3. General treatment reactions and withdrawals from the study

Patient No.	Dose, BU/ml $\times$ 1,000	Reaction	Treatment	Withdrawal
1	100	rash, conjunctivitis and wheezing; 5-week interval	timothy	no
2	60–100	rash, conjunctivitis and wheezing	timothy	yes
3	dose increase 5–25!	mild	<i>D. farinae</i>	no
4	4	moderate wheezing, flushing and rash; 1-month interval	<i>D. farinae</i>	no
5	0.1	mild	<i>D. farinae</i>	yes
6	0.5	mild	<i>D. farinae</i>	yes
7	dose increase 2–10!	mild	<i>D. farinae</i>	no
8	0.2	moderate wheezing, flushing and rash; first visit	<i>D. farinae</i>	yes

Statistics

Before analysis, CPT threshold concentrations and in vitro titres were logarithmically transformed to obtain normal distribution of the data.

For estimation of the difference in CPT threshold concentrations and allergen-specific s-IgG titres between the groups at different times, i.e. at 0, 1, 2 and 3 years, and to test the change from before to after 1, 2 and 3 years of SCIT, the Kolmogorov-Smirnov test was used. For estimation of changes within groups over time, i.e. from before to after 1, 2 and 3 years of SCIT, the Wilcoxon rank sum test was used.

Data were also analyzed by statistical methods for repeated measures. Univariate analysis of variance accounting for correlations between measurements on the same subject was performed [12]. Relevant contrasts were calculated. For calculations, the SAS procedure PROC MIXED was used [13].

To evaluate the self-assessment of symptoms after 3 years of SCIT compared to prior to SCIT with 5 classes, i.e. from 1 (much better) to 5 (much worse), the level of symptoms before treatment was given the value 3 and then compared to the estimate by patients after 3 years of SCIT using the Wilcoxon rank sum test.

p values less than 0.05 were regarded statistically significant.

Results

Patients

Of the initial 20 patients, 6 dropped out of the study. Four withdrew following mild systemic reactions (1 given timothy and 3 given *D. farinae* extract; table 3), 1 was withdrawn because of poor compliance and 1 emigrated. Thus, a total of 2 patients from the timothy group and 4 from the *D. farinae* group did not complete the study. Fourteen patients remained on treatment for 3 years, 7 on *D. farinae* and 7 on timothy SCIT.

Immunotherapy

All patients receiving timothy SCIT reached the targeted top dose of 100,000 BU, containing approximately

100 μ g of major allergen. However, among those receiving *D. farinae* allergen extract, only 1 patient reached 100,000 BU, while 1 reached 90,000 BU, 2 reached 80,000 BU and 1 patient each reached 7,000, 6,000 and 4,000 BU, respectively.

Conjunctival Provocation Tests

For timothy-treated patients, the timothy CPT threshold concentration increased from a mean of 6,166 BU/ml [95% confidence interval (CI) 676–54,954] before SCIT to 100,000 BU/ml (95% CI 12,023–831,764) after 3 years of SCIT, i.e. a 16.2-fold increase ($p < 0.05$), whereas in mite-treated patients the increase was 3.7-fold. The difference in the change within treatment groups was significant ($p < 0.05$; table 4a).

Similarly, in patients given *D. farinae* SCIT, the mite CPT threshold concentration increased from 724 BU/ml (95% CI 219–2,344) to 26,915 BU/ml (95% CI 2,042–346,737), i.e. a 32-fold increase ($p < 0.05$), whereas in timothy-treated patients, the mite CPT threshold concentration increased only 3.7-fold. The difference in the change within groups was significant over 3 years ($p < 0.05$; table 4a).

The difference in the change between treatment groups was significant for *D. farinae* CPT after 2 and 3 years of SCIT and for timothy CPT after 1, 2 and 3 years of SCIT ($p < 0.05$; fig. 1a, b).

The overall assessment of the change in allergen CPT threshold concentration including all data (95 degrees of freedom) was highly significant in favour of the allergen used for treatment ($p < 0.015$).

Allergen-Specific IgG Antibody Levels

Most patients in both groups had low levels of anti-grass s-IgG levels at the start of immunotherapy (table 1); 11 patients had no detectable anti-grass s-IgG antibodies.

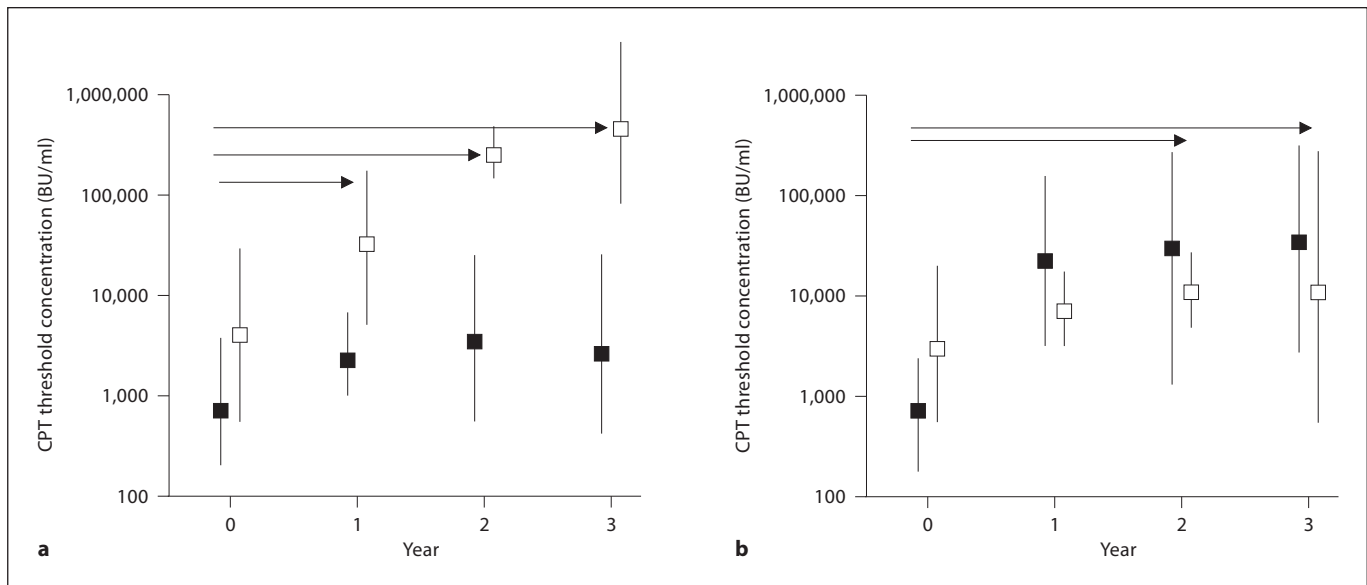


Fig. 1. The CPT threshold concentrations for timothy allergen extract (a) and *D. farinae* allergen extract (b) after the pre-treatment season (year 0) and after 1, 2 and 3 years of immunotherapy with *D. farinae* (filled squares) or timothy (open squares) allergen extract. Means and 95% CIs are shown. Significant differences in the change in CPT threshold concentration between groups from the pre-treatment year to after 1, 2 and 3 years of immunotherapy are indicated by black arrows ($p < 0.05$, Kolmogorov-Smirnov test for differences between groups).

Table 4a. Changes in CPT threshold concentration after 1, 2 and 3 years of treatment compared to baseline within treatment groups (mean fold change)

SCIT		Change in CPT threshold concentration			
		timothy		<i>D. farinae</i>	
		fold change	p	fold change	p
1 year	<i>D. farinae</i>	2.3	n.s.	13.8	0.018
	timothy	5.1	0.043	3.7	0.043
2 years	<i>D. farinae</i>	3.2	n.s.	26.9	0.028
	timothy	31.6	0.028	4.4	n.s.
3 years	<i>D. farinae</i>	2.1	n.s.	37.2	0.018
	timothy	16.2	0.018	3.7	0.043

n.s. = Not significant.

Table 4b. Changes in allergen-specific IgG concentrations after 1, 2 and 3 years of treatment compared to baseline within treatment groups (mean fold change)

SCIT		Change in allergen-specific IgG concentration			
		timothy		<i>D. farinae</i>	
		fold change	p	fold change	p
1 year	<i>D. farinae</i>	-0.67	n.s.	3.6	0.018
	timothy	2.2	0.028	-0.52	n.s.
2 years	<i>D. farinae</i>	-0.89	n.s.	3.8	0.018
	timothy	4.9	0.018	-0.77	n.s.
3 years	<i>D. farinae</i>	1.2	n.s.	3.2	0.043
	timothy	4.4	0.018	-0.58	n.s.

n.s. = Not significant.

However, a majority of patients (10/14) had high levels of anti-mite s-IgG antibodies before starting the study, probably due to exposure to high levels of *D. farinae* allergens in the environment.

For grass-treated patients, the timothy allergen-specific IgG antibody titres increased from a mean of 19.8 U/ml (95% CI 0–59.8) before SCIT to 86.5 U/ml (95% CI

34.1–138.9) after SCIT, i.e. a 4.4-fold increase ($p < 0.018$), and the *D. farinae* allergen-specific s-IgG antibody titres changed from 48 U/ml (95% CI 23–73.5) to 154.7 U/ml (95% CI 29.9–279.5), i.e. a 3.2-fold increase ($p < 0.043$), for mite-treated patients (table 4b).

The difference in the change in anti-timothy and anti-*D. farinae* IgG antibody levels from before to after 1, 2 and

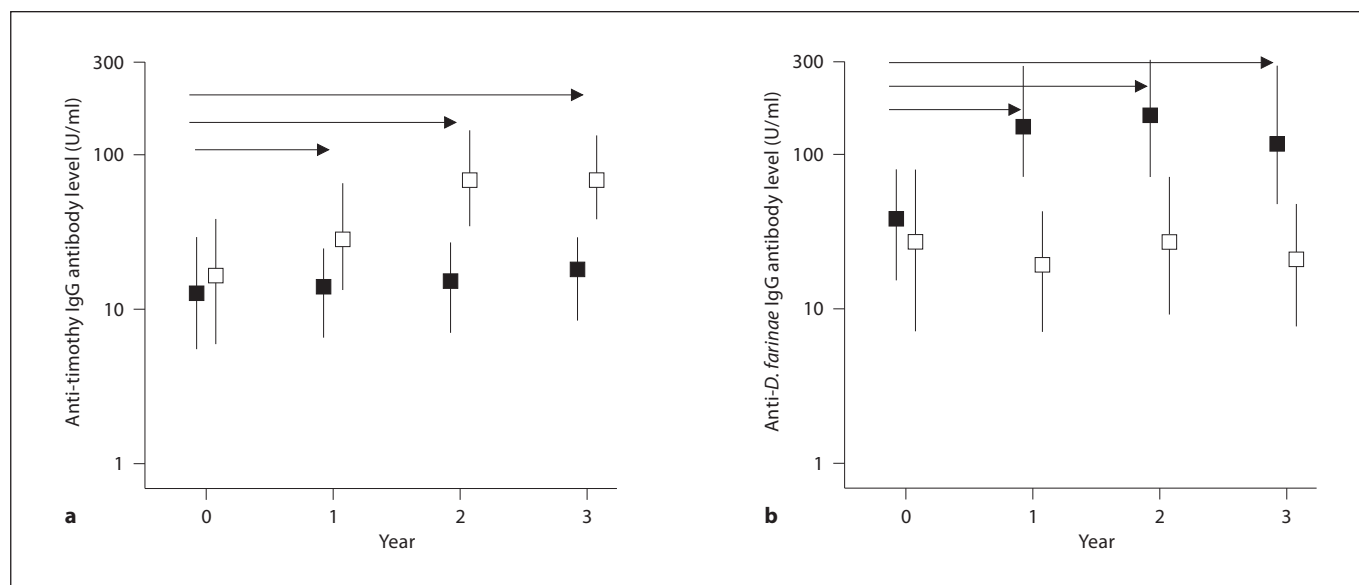


Fig. 2. The level of anti-timothy IgG antibody titres (a) and anti-*D. farinae* IgG antibody titres (b) after the pre-treatment season (year 0) and after 1, 2 and 3 years of immunotherapy with *D. farinae* (filled squares) or timothy (open squares) allergen extract. Means and 95% CIs are shown. Significant differences in the change in IgG antibody titres between groups from the pre-treatment year to after 1, 2 and 3 years of immunotherapy are indicated by black arrows ($p < 0.05$, Kolmogorov-Smirnov test for differences between groups).

3 years of treatment was significant comparing the timothy and *D. farinae* (placebo) groups ($p < 0.05$; fig. 2a, b).

The overall assessment of the change in allergen-specific s-IgG antibody titres including all data (95 degrees of freedom) was highly significant in favour of treatment ($p < 0.0001$).

Global Assessments

Global Evaluation of Change in Symptoms Compared to before Treatment

The patients on active treatment improved during their season, i.e. timothy-treated patients ($p = 0.041$) and *D. farinae*-treated patients ($p = 0.018$) during the summer and autumn, respectively. There was no significant change in perceived improvement between groups, likely reflecting, at least in part, the low numbers studied.

Side Effects

Eight general reactions occurred in as many patients, 2 among the timothy-treated group and 6 in the group receiving *D. farinae* SCIT (table 3).

Two patients in the timothy group had moderate reactions with a rash, conjunctivitis and wheezing, 1 on maintenance treatment with 100,000 BU/ml reconstituted with Depot diluent, the other when the dose was in-

creased from 60,000 to 100,000 BU/ml with the extract reconstituted with Albumin diluent. The latter patient withdrew from the study. Mild reactions occurred in 2 patients in the *D. farinae* group after 100 and 500 BU, respectively (both withdrew), and in 2 others at 10,000 and 25,000 BU. The latter was mistakenly given a dose 5 times higher than the planned dose, i.e. 25,000 instead of 5,000 BU. Two patients on *D. farinae* SCIT had moderate reactions with wheezing, flushing and a rash after 200 BU (patient withdrew) and 4,000 BU, respectively.

Discussion

This study was performed during the early 1980s, at a time when there were few double-blind placebo-controlled studies on the efficacy of allergen immunotherapy/vaccination. Thus, the primary goal was to explore the efficacy of immunotherapy. However, diary symptom scores (data not shown) did not show efficacy, and the study was not published on the basis that it was inadequately powered for this endpoint. The reasons for publishing the trial now, i.e. 30 years later, are fourfold.

Firstly, this is the first double-blind study showing that 'specific' allergen immunotherapy, as judged by provoca-

tion testing in the target organ, is allergen-specific. There are very few previous studies that have explored the allergen specificity of SCIT [3–8].

In 1978, Norman and Lichtenstein [3], in a placebo-controlled double-blind study, gave ragweed SCIT to a group of patients with grass and ragweed hay fever, scoring symptoms during both seasons. Symptoms improved during the ragweed season but not during the grass pollen season.

Lack et al. [4] aimed to determine the immunologic consequences of rush immunotherapy. In an open study, they prospectively studied 10 patients who had positive skin test responses to house dust mite *D. pteronyssinus* and cat dander extract. All patients received rush immunotherapy with mite extract but not cat dander extract over 2–4 weeks. In all patients, they found a significant reduction in skin and nasal reactivity to mite allergen but not to cat allergen, accompanied by a rise in serum anti-*D. pteronyssinus* IgE and serum anti-*D. pteronyssinus* IgG4 levels, whereas anti-cat IgE was not altered in 7 out of 7 patients.

Winther et al. [6] used a comparable design to that of our study; i.e. they treated birch- and timothy pollen-allergic patients with either extract in a double-blind manner. However, due to low pollen counts at follow-up, differences between birch- and timothy-treated patients were found only during the birch pollen season. Furthermore, target organ sensitivity was not examined.

The second reason to publish our results at this time is to point out the possibility of using another allergen, to which the patients are clinically sensitive, as ‘placebo’ instead of a histamine placebo. Preliminary studies of subcutaneous injection of different concentrations of histamine had revealed that the sensation elicited in patients when histamine and allergen were injected subcutaneously differed, thereby compromising the blinding of the study. In the present trial, no patient was able to determine whether timothy or *D. farinae* extract was injected. Thus, this method can be recommended as an alternate design in future double-blind studies with allergen immunotherapy.

The third reason to discuss our results is the fact that, although the number of patients was low, we found significant and convincing results using conjunctival challenge tests. We acknowledge the limitations of the small number of subjects studied. Fourteen out of 20 participants completed the 3-year study, which is equivalent to a drop-out rate of around 10% per year. In this early trial, we used maximum doses that were at least 10 times higher than has been used in trials performed since 1998 [2].

It was this fact that made it possible to show convincing differences in changes in CPT threshold concentration and increased allergen-specific s-IgG titres in the active groups in relation to the placebo groups despite the low numbers studied. While the study demonstrated clear-cut allergen specificity by conjunctival testing, there was insufficient power to detect between-group differences for symptoms upon natural exposure to the relevant allergen. The lack of difference for symptoms between groups could also be explained in part by continuous mite exposure in the United Kingdom during the pollen season, albeit at low levels, and persistent nasal airway responsiveness and symptoms during the autumn months following ‘priming’ during seasonal pollen exposure. Our findings of within-group differences for symptoms and both within- and between-group differences for conjunctival allergen sensitivity emphasise that single allergen immunotherapy affords protection against the relevant allergen in polysensitised patients, as confirmed in other studies [for example 14].

The fourth reason to publish now is certainly the 100-year anniversary of the first publication on allergen immunotherapy by Noon in 1911 [1]. Noon also relied on conjunctival testing to demonstrate successful desensitisation. Here, we use the same method under blinded conditions to illustrate the allergen specificity of immunotherapy.

In conclusion, we present convincing evidence that SCIT is allergen-specific and that the inclusion of an alternative allergen as an active ‘placebo’ control in SCIT trials is useful. This approach should be considered an acceptable alternative for future immunotherapy trials using both SCIT and sublingual immunotherapy.

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