PREVENTION OF NEW SENSITIZATIONS IN MONOSENSITIZED SUBJECTS SUBMITTED TO SPECIFIC IMMUNOTHERAPY OR NOT. A RETROSPECTIVE STUDY


This was an open, retrospective study. A total of 8,396 mono-sensitized patients with respiratory symptoms were selected for either Group A (7,182) who were given immunotherapy for 4 years and then treated with medication for 3 years or Group B (1, 214) who were treated with medications only for 7 years. All patients were skin prick tested with standard allergens. All patients also underwent specific IgE before and 4 years after treatment with repeat examination 3 years later. “Poly-sensitized patients were 23.75% in Group A and 68.03% in Group B after 4 years ($P<0.0001$) and 26.95% and 76.77%, respectively, after 7 years ($P<0.0001$).” Asthmatic patients were more prone to develop polysensitization when compared to subjects who only suffered from rhinitis (32.14% instead of 27.9% after 4 years, 36.5% instead of 31.33% after 7 years; ($P<0.0001$). Total IgE decreased by 17.53% in Group A but increased by 13.71% in Group B ($P<0.0001$). This study showed a reduction in new sensitizations in mono-sensitized patients with respiratory allergic disease.
Prevention of new sensitizations in monosensitized subjects submitted to specific immunotherapy or not. A retrospective study

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Summary

Background Specific immunotherapy is the only currently available allergen-orientated treatment able to modify the natural history of respiratory allergic diseases. Safety and clinical efficacy of this treatment are well documented, but evidence about the ability to reduce new sensitizations is still poor. Objective We report a retrospective study conducted in order to assess the prevention of new sensitizations in monosensitized subjects treated with specific immunotherapy vs. monosensitized patients treated with anti-allergic drugs. Methods 8396 monosensitized patients with respiratory symptoms were selected according to an open, retrospective design. Group A included 7182 patients submitted to specific immunotherapy (and anti-allergic drugs when needed) for 4 years and then treated with drugs for at least 3 years. Group B included 1214 patients treated only with drugs for at least 7 years. All patients underwent prick test with a standard panel of allergens and total and specific IgE determination before and after 4 years of treatment and again 3 years later. Results Groups were well balanced. Polysensitized subjects were 23.75% in Group A and 68.03% in Group B after 4 years ($P < 0.0001$) and 26.95% and 76.77%, respectively, after 7 years ($P < 0.0001$). Asthmatic subjects were more prone to develop polysensitization in comparison to subjects suffering only from rhinitis (32.14% instead of 27.29% after 4 years, 36.5% instead of 31.33% after 7 years; $P < 0.0001$). Specific IgE decreased by 24.11% in Group A and increased by 23.87% in Group B ($P < 0.0001$). Total IgE decreased by 17.53% in Group A and increased by 13.71% in Group B ($P < 0.0001$). Conclusions Specific immunotherapy was observed retrospectively to reduce new sensitizations in monosensitized subjects suffering from respiratory allergic diseases.

Keywords: allergy, monosensitization, polysensitization, prevention, respiratory symptoms, specific immunotherapy

Introduction

Allergen-specific immunotherapy (SIT) was first used in medical practice at the beginning of the last century [1] for the treatment of patients suffering from respiratory allergic diseases. The efficacy and safety of SIT has been
questioned until recently. In 1998 the WHO Position Paper [2] and the EAAci Position Paper [3], on the basis of a careful revision of the available literature, stated the safety and efficacy of SIT performed by injections or by local (nasal, sublingual) administrations under adequate conditions. Further studies supporting efficacy and safety of both injective and local SIT have been published afterwards [4–11].

A long-term effect of SIT after a 3-year treatment has also been reported [12–15].

Several studies run in the last decades have been dedicated to the natural history of the respiratory allergic diseases, showing its evolution towards asthma and polysensitization.

Because SIT is able to modify the immune response [2,3], a modification of the natural history after such treatment in patients suffering from allergic rhinitis can be expected but the assessment requires a large number of carefully selected patients and a very long follow-up. Only a few papers have documented the ability of SIT to prevent or at least delay the development of asthma in patients suffering from allergic rhinitis [16–18].

The ability of SIT to prevent the development of new sensitizations in monosensitized patients has been so far documented in only one paper involving 44 children [19].

We therefore designed a protocol to study retrospectively the development of polysensitization in a large population of monosensitized patients submitted to SIT for 4 years in comparison to a parallel group of monosensitized patients treated with anti-allergic drugs only.

Materials and methods

Study design

The study was planned as a retrospective parallel group open study including patients suffering from allergic rhinitis and/or asthma followed-up as outpatients in the period 1980–99.

Inclusion criteria

Patients were considered eligible for the study under the following conditions:

- age at least 14 years
- clinical history (at least one year) of allergic rhinitis and/or mild to moderate asthma (symptoms from at least twice a week to daily; FEV1 from not below 80% to at least 70% predicted)
- monosensitization to one of the following respiratory allergens: Dermatophagoides farinae, Dermatophagoides pteronyssinus, Compositae mix (Artemisia, Ambrosia), Corylaceae-Betulaceae mix (Alder, Birch, Hazel), grass mix, Olea europaea, Parietaria judaica
  - positive skin prick test weal to the relevant allergen (≥ histamine 10 mg/mL)
  - in vitro specific IgE to the relevant allergen ≥ class 4

Study groups

Patients were subdivided into Group A; including patients submitted to SIT, and Group B; including patients not submitted to SIT.

Each Group was further subdivided into two subgroups, including patients suffering only from rhinitis without any asthmatic symptom at enrolment (A1 and B1), and patients who had clear asthmatic symptoms with or without rhinitis at enrolment (A2 and B2).

SIT was proposed after diagnosis to all patients, but was accepted only by patients subsequently included in Group A. Patients unavailable to accept SIT (mainly for economical or compliance reasons) were included in Group B.

Skin prick tests

All patients attending our clinic, suffering from respiratory symptoms and sensitized to at least one inhalant allergen, have been routinely submitted to skin prick tests 4 years (T1; end of SIT for Group A) and 7 years (T2) after the first diagnosis (T0). The skin prick tests were performed by trained allergologists in our centre according to a routine procedure and using a standard panel of respiratory allergens including mites (D. farinae and D. pteronyssinus), Parietaria judaica, grass mix, Compositae mix (Artemisia and Ambrosia), Corylaceae-Betulaceae mix (Alder, Birch, Hazel), Olea europaea, Alternaria tenuis, positive and negative control. Tests were done out of the season in
pollen-sensitized patients and in symptom-free periods in patients sensitized to mites. All patients were instructed not to take drugs during the 3 weeks before the test.

Commercial allergenic extracts from different suppliers have been used during the investigation here described.

**Total and specific IgE**

A sample of blood was taken from each patient at T0 and T2 for the *in vitro* determination of total IgE and allergen-specific IgE according to a routine procedure followed in our centre.

Different analytical methods, according to what was state-of-the-art at each period, have been used during the investigation (RAST, ELISA).

**Drugs**

All patients, whether treated with SIT or not, were prescribed and instructed to use the same anti-allergic drugs for the control of the respiratory symptoms.

Different drugs have been used during the study period, according to their commercial availability. Patients were administered antihistamine tablets, inhaled B-2 adrenergic agonists, and topical and systemic steroids.

**Environmental avoidance measures**

All patients positive to mites, whether submitted to SIT or not, have been instructed to take standard environmental measures to decrease the allergenic load (i.e. no use of humidifiers, frequent vacuum cleaning, washing sheets with water at $\geq 55^\circ C$ at least once a week and removal of carpets, soft toys and plants from the patient’s bedroom).

**Statistics**

A parametric test (*t*-test) was used for total and specific IgE statistical analysis. Chi-square test for independent data was used for analysis of rates between groups.

$P$-values $< 0.05$ and $< 0.01$ were considered, respectively, as statistically significant and highly statistically significant.

Statistical analysis was done with a standard statistical package (BMDP, Los Angeles, CA, USA)

**Results**

**Demographic data**

A total of 8396 patients monosensitized to mites, grass, olive, *Compositae*, *Corylaceae-Betulaceae* or *Parietaria* have been selected for the study.

Groups and subgroups were well balanced for sex and pathology (Table 1).

**Sit**

Allergens used for SIT are summarized in Table 2.

Most patients (4018/7182, 55.95%) were treated with pollen extracts, mainly represented by *Parietaria judaica* (3092/4018, 76.95% of patients submitted to SIT with pollen extracts).

Mite extracts were administered for SIT to 3164/7182

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Table 1. Demographic data of patients (T0)

<table>
<thead>
<tr>
<th></th>
<th>Number of patients</th>
<th>Mean age (years)</th>
<th>Female (%)</th>
<th>Male (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SIT</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Subgroup A1 (rhinitis)</td>
<td>2938</td>
<td>22.96</td>
<td>1657 (56.4)</td>
<td>1281 (43.6)</td>
</tr>
<tr>
<td>Subgroup A2 (asthma and rhinitis)</td>
<td>4244</td>
<td>23.45</td>
<td>2351 (55.4)</td>
<td>1893 (44.6)</td>
</tr>
<tr>
<td>Group A (total)</td>
<td>7182</td>
<td>23.25</td>
<td>4008 (55.8)</td>
<td>3174 (44.2)</td>
</tr>
<tr>
<td><strong>DRUGS</strong></td>
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</tr>
<tr>
<td>Subgroup B1 (rhinitis)</td>
<td>499</td>
<td>21.56</td>
<td>285 (57.1)</td>
<td>214 (42.9)</td>
</tr>
<tr>
<td>Subgroup B2 (asthma and rhinitis)</td>
<td>715</td>
<td>23.03</td>
<td>407 (56.9)</td>
<td>308 (43.1)</td>
</tr>
<tr>
<td>Group B (total)</td>
<td>1214</td>
<td>22.42</td>
<td>692 (57.0)</td>
<td>522 (43.0)</td>
</tr>
</tbody>
</table>
patients (44.05%), whereas grass extracts were administered to 817/7182 patients (20.33%).

Parietaria, mites and grass extracts together accounted for more than 98% of SIT.

Development of new sensitizations

Only 1706 out of 7182 (23.75%) monosensitized patients treated for four years with SIT (Group A) showed at least one new sensitization as compared to 826 out of 1214 (68.03%) patients of the parallel group (Group B) who developed new sensitizations whereas, 3 years later, 1936/7182 (26.95%) patients in Group A and 932/1214 (76.77%) patients in Group B developed new sensitizations (Table 3). Both comparisons were highly significant ($P \leq 0.0001$, Table 4, upper part).

Patients suffering from only rhinitis, treated with SIT or not, appeared significantly less prone to develop new sensitizations 4 or 7 years after the first observation ($P \leq 0.0001$ to $P = 0.0086$ for all comparisons; Table 4, upper part).

Patients submitted to SIT during the previous four years were, with a high statistical significance, less prone to develop new sensitizations during the following three years in comparison to patients treated only with drugs (A vs. B, $P \leq 0.0001$; Table 4, lower part); in fact, 230 patients out of 5476 (4%) patients still monosensitized after 4 years of SIT showed at least one new sensitization after 3 years as compared to 106/388 (27.32%) patients of the drug group.

This was also true for patients suffering from only rhinitis submitted to SIT or not (A1 vs. B1) and for patients suffering from asthma and rhinitis submitted or not to SIT (A2 vs. B2) in the same period ($P \leq 0.0001$ for both; Table 4, lower part).

According to the calculated average annual rate of

<table>
<thead>
<tr>
<th>Genus or Group</th>
<th>Number of patients (%)</th>
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<tbody>
<tr>
<td>Seasonal allergens</td>
<td></td>
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<tr>
<td>Parietaria</td>
<td>3092 (76.95)</td>
</tr>
<tr>
<td>Grass</td>
<td>817 (20.33)</td>
</tr>
<tr>
<td>Olea</td>
<td>57 (1.42)</td>
</tr>
<tr>
<td>Compositae (mix)</td>
<td>28 (0.70)</td>
</tr>
<tr>
<td>Corylaceae-Betulaceae (mix)</td>
<td>24 (0.60)</td>
</tr>
<tr>
<td>Total pollens</td>
<td>4018 (55.95)</td>
</tr>
<tr>
<td>Perennial allergens</td>
<td></td>
</tr>
<tr>
<td>Mites</td>
<td>3164 (44.05)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Genus or Group</th>
<th>Number of patients (%)</th>
</tr>
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</table>

### Table 2. Allergens used for SIT

### Table 3. Polysensitized patients after 4 (T1) and 7 (T2) years

### Table 4. Statistics on the evolution of polysensitization

Comparison between groups or subgroups (absolute values at T1 and T2) $P$-value

<table>
<thead>
<tr>
<th></th>
<th>T1</th>
<th>T2</th>
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<tbody>
<tr>
<td>A vs. B</td>
<td>$&lt; 0.0001$</td>
<td>$&lt; 0.0001$</td>
</tr>
<tr>
<td>A1 vs. A2</td>
<td>$&lt; 0.0001$</td>
<td>$&lt; 0.0001$</td>
</tr>
<tr>
<td>B1 vs. B2</td>
<td>0.0086</td>
<td>0.0002</td>
</tr>
<tr>
<td>A1 vs. B1</td>
<td>$&lt; 0.0001$</td>
<td>$&lt; 0.0001$</td>
</tr>
<tr>
<td>A2 vs. B2</td>
<td>$&lt; 0.0001$</td>
<td>$&lt; 0.0001$</td>
</tr>
<tr>
<td>(A1 + B1) vs. (A2 + B2)</td>
<td>$&lt; 0.0001$</td>
<td>$&lt; 0.0001$</td>
</tr>
</tbody>
</table>

Comparison between groups or subgroups (evolution from T1 to T2) $P$-value

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<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>A vs. B</td>
<td></td>
<td>$&lt; 0.0001$</td>
</tr>
<tr>
<td>A1 vs. A2</td>
<td>0.043</td>
<td></td>
</tr>
<tr>
<td>B1 vs. B2</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>A1 vs. B1</td>
<td></td>
<td>$&lt; 0.0001$</td>
</tr>
<tr>
<td>A2 vs. B2</td>
<td>$&lt; 0.0001$</td>
<td></td>
</tr>
<tr>
<td>(A1 + B1) vs. (A2 + B2)</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

Group A: SIT; Group B: drugs; Subgroups A1 and B1: rhinitis only; Subgroups A2 and B2: asthma and rhinitis
development of new sensitizations from 0 to 4 years, 1 out of 4 (24.8%) monosensitized patients treated with drugs as compared to only 1 out of 15 (6.6%) monosensitized patients treated with SIT developed a new sensitization per year in this period.

The average annual rate of new sensitizations from 4 to 7 years was lower in value but still higher in patients treated with drugs as compared to patients treated with SIT (1 out of 10, i.e. 10.1% and 1 out of 72, i.e. 1.4%, respectively) and also higher in asthmatic patients as compared to patients with rhinitis.

Table 5. Specific and total IgE (kU/L)

<table>
<thead>
<tr>
<th></th>
<th>Specific IgE mean value (SD)</th>
<th>Total IgE mean value (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T2</td>
</tr>
<tr>
<td>SIT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subgroup A1 (rhinitis)</td>
<td>40.501  (15.804)</td>
<td>31.319  (12.788)</td>
</tr>
<tr>
<td>Subgroup A2 (asthma and rhinitis)</td>
<td>44.009 (16.635)</td>
<td>33.011 (13.016)</td>
</tr>
<tr>
<td>Group A</td>
<td>42.576 (16.391)</td>
<td>32.319 (12.949)</td>
</tr>
<tr>
<td>DRUGS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subgroup B1 (rhinitis)</td>
<td>38.507  (15.425)</td>
<td>48.902 (16.975)</td>
</tr>
<tr>
<td>Subgroup B2 (asthma and rhinitis)</td>
<td>41.202 (13.789)</td>
<td>50.201 (13.915)</td>
</tr>
<tr>
<td>Group B</td>
<td>40.094 (14.538)</td>
<td>49.667 (15.254)</td>
</tr>
</tbody>
</table>

Group A: SIT; Group B: drugs; Subgroups A1 and B1: rhinitis only; Subgroups A2 and B2: asthma and rhinitis

Total and allergen-specific IgE

Average values for each Group and subgroup at time T0 and T2 are shown in Table 5. Allergen-specific IgE were significantly higher in Group A and subgroups as compared to the corresponding Group B and subgroups at T0 (P = 0.009 for A1 compared to B1; P < 0.0001 for other comparisons).

Total IgE were similar at T0 in Group A and B (P = 0.682), and also in subgroups (A1 vs. B1, P = 0.437; A2 vs. B2 P = 0.235).

Allergen-specific IgE decreased significantly in Group A (-24.11%) and subgroups (-22.63% for A1; -25.00% for A2) and increased significantly in Group B (+23.87%) and subgroups (+27.00% for B1; +21.84% for B2) after 7 years (P < 0.0001 in all cases).

Total IgE paralleled the trend already shown for specific IgE in Group A (-17.53%) and Group B (+13.71%) and subgroups (-16.69% for A1 and -18.09% for A2; +11.56% for B1 and +15.19% for B2) in all cases with a high statistical significance (P < 0.0001).

Because of these differential trends, specific and total IgE in group and subgroups A were significantly lower at T2 as compared to the corresponding B Group and subgroups (P < 0.0001 in all cases).

Patients suffering from asthma and rhinitis at enrolment had higher specific and total IgE in comparison to patients belonging to the same group and suffering only from rhinitis, before and after the treatment. The difference was highly significant at T0 and T2 for specific and total IgE in patients belonging to Group A (P < 0.0001). For patients belonging to Group B the difference was highly significant at T0 for specific IgE (P = 0.002) but not for total IgE (P = 0.475). In the same Group at T2 the difference was not significant for specific IgE (P = 0.140) and significant (P = 0.028) for total IgE.

Patients submitted to SIT and polysensitized after 7 years (1936/7182) had significantly higher levels of both specific and total IgE at T0 when compared to patients still monosensitized at T2 (239.52 as compared to 191.18, P < 0.0001 for specific IgE; 52.80 as compared to 38.78, P < 0.0001 for total IgE).

Discussion

Because cat was not included in our standard prick test panel of allergens at the beginning of the period considered in our study, we excluded cat monosensitized patients from...
our analysis. Patients monosensitized to Alternaria were also excluded because of the low reliability until recent years of this extract [20–22].

Patients monosensitized to Parietaria judaica are largely represented in our survey because this pollen is the main one in our region (Sicily, Mediterranean Coast of Southern Italy).

Allergens and materials for in vivo and in vitro assessments have undergone substantial improvement during the last two decades but the large number of patients included in both groups of our study over the same period of time is expected to compensate for the possible differences due to such unavoidable variations. In other words, absolute rates could have been overestimated, but comparisons between parallel groups are expected to give reliable results.

Many longitudinal studies have focused on the increase of the sensitization rate from childhood to adulthood [23–26], but only three recent reports analysed the evolution from mono to polysensitization. One paper reached the conclusion that the relative increase in polysensitized patients was age-related [27]. A further paper by the same investigators assessed the development of polysensitization in 72 out of 165 (43.6%) previously monosensitized children after 2–10 years from the first diagnosis [28]. Both studies were run on children treated only with drugs.

Children monosensitized to mites (age below 6 years) were studied for the development of polysensitization in one double-blind placebo-controlled study [9]. All patients belonging to the control group (22 children) developed at least one new sensitization after 3 years as compared to only 10 children belonging to the active group including 22 children. Our results are in good agreement with the three papers mentioned above taking into consideration that the mean age of our subjects was 22.42 years.

The efficacy of SIT is no longer a matter of debate [2,3], but the cost/benefit ratio can be further improved if this therapeutical approach turns out to be able to prevent both the development of asthma and further sensitizations.

We believe that our data, obtained in two very large and well-balanced groups of patients, strongly support this last point. Furthermore, differently from studies run in highly selected groups of patients with treatments prepared ad hoc and administered according to special schedules, our results have been obtained with commercially available allergen preparations administered according to standard schedules to patients not selected according to special criteria.

Patients submitted to SIT showed a significantly lower number of new sensitizations not only during the 4-year treatment, but also during the 3-year period after its interruption, as compared to the parallel group of patients treated only with drugs.

These results mean that SIT has a preventive effect towards new sensitizations, not only while it is performed, but for at least three years after its interruption.

New sensitizations were significantly more likely to take place in patients suffering from asthma and rhinitis as compared to patients suffering only from rhinitis.

The average annual rates of new sensitizations in the period 0–4 years were higher than values from 4–7 years (6.6% and 1.4% in Group A and 24.8% and 10.1% in Group B, respectively). In our opinion, this means that there is an early risk to develop a new sensitization after the first one. Because SIT-treated patients develop new sensitizations at a 4-fold lower average annual rate from 0–4 years and at a 7-fold lower average annual rate from 4–7 years than patients treated with drugs (6.6% vs. 24.8% and 1.4% vs. 10.1%, respectively), SIT should be considered very early after the first sensitization has taken place to prevent further sensitization(s).

These results in the SIT group are somewhat surprising because a lower average annual rate should have been expected during instead of after SIT. According to our data, SIT is only able to decrease the absolute rate but not the normal trend towards new sensitizations. In our opinion, this point deserves further investigation.

Data about specific and total IgE are in good agreement with the observed trend of polysensitization.

Patients belonging to Group A and subgroups (SIT) were well balanced with patients of Group B and subgroups for total IgE but not for specific IgE. The absolute difference between groups was small (6.16%) but nonetheless significant because of the high number of samples analysed. This means that Group A started from a worse condition than Group B if we consider specific IgE concentration as a signal of the clinical condition of patients.

Patients suffering only from rhinitis had significantly lower specific and total IgE levels than patients suffering from asthma and rhinitis at T0 in both Groups. This again stresses the link between IgE concentration and the clinical condition of patients.

After 4 years of SIT and 3 years of drugs patients showed a sharp decrease of both specific and total IgE (-24.11% and -17.53%, respectively), as compared to a sharp increase of the same two parameters in patients treated only with drugs for 7 years (+23.87% and +13.71%, respectively). The decrease of specific and total IgE was higher in patients submitted to SIT and suffering from asthma and rhinitis as compared to those suffering only from rhinitis. A decrease in specific and total IgE is a common finding in SIT studies and therefore our data are in good agreement with previous experiences.

Patients belonging to the SIT group who developed new sensitizations at T2 had initially higher levels of specific
and total IgE as compared to patients still monosensitized at T2 (+25.11% and +36.15%, respectively). According to our data, an increase of both specific and total IgE in Group B (only drugs) was paralleled by an higher rate of new sensitizations, while the decrease of both specific and total IgE in Group A (SIT) was accompanied by a lower rate of new sensitizations.

It is known that SIT is able to decrease IL-4 production by peripheral blood mononuclear cells (PBMC), in this way decreasing both Th2 differentiation and IgE production [29]. SIT has also been shown capable of reducing IL-5 and IgE production after stimulation with Der f 1, and this reduction parallels the clinical outcome of the treatment [30]. The induction of tolerance to the allergen [31] and the development of a state of specific anergy in peripheral T cells by IL-10 [32] are other immunological changes associated with SIT. Polysensitized patients have been shown able to produce higher levels of IL-4 after a specific stimulation as compared to monosensitized patients [33] and they also showed higher levels of both specific and total IgE [34]. On the other hand, local production of IgE in the lung lasting 7–14 days after one antigenic challenge has been shown [35] and the same event can be expected during the natural exposure to the relevant allergen(s).

Our results are in agreement with, and give further support to, these findings.

Monosensitized patients suffering from asthma and rhinitis, with high levels of specific and total IgE, and not submitted to SIT were the best candidates for polysensitization as opposed to patients suffering only from rhinitis with low levels of specific and total IgE and submitted to SIT. Moreover, monosensitized patients not treated with SIT were candidates to specific and total IgE levels increase.

As in our clinical experience, SIT seems to be less effective in polysensitized patients and in patients suffering from asthma and rhinitis. In our opinion, SIT should be performed as early as possible in monosensitized patients suffering only from rhinitis to prevent polysensitization and the development of asthma. Similar experiences on the preventive effects of SIT in reducing the onset of further sensitizations have been reported only in children [36,37] as well as the preventive effect on the development of asthma [17,18].

Data about the development of asthma in our personal experience, not considered in this paper, will be dealt with in another report.

We believe that the results here presented, obtained in a very large sample of patients followed up for at least seven years, are an important contribution to the current knowledge about the outcomes of specific immunotherapy in reducing the onset of further sensitizations in monosensitized patients who undergo this kind of treatment.

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LONG-TERM CLINICAL EFFICACY OF GRASS-POLLEN IMMUNOTHERAPY


A randomized, double-blind, placebo-controlled, three year trial in which patients who had been on immunotherapy 3-4 years, were discontinued. Seasonal symptoms and use of rescue medications were the scores used as primary outcome measures. Immediate and late skin responses as well as immediate conjunctival response to allergen challenge were the objective measures used. At twenty-four hours, punch biopsy specimens were obtained after intradermal injection challenge and were examined for T-cell infiltration and cytokine-producing T helper cells. In addition, another group of hay fever sensitive patients who had not received subcutaneous immunotherapy were followed. Seasonal symptom scores as well as rescue anti-allergic medication scores remained low for the group on immunotherapy as well as for those who were discontinued. Scores for both groups were lower than those who had not taken immunotherapy. Clinical improvement as well as a decrease in the late skin response to the grass pollen challenge, blunting of CD3+T-cell infiltrate, and fewer interleukin 4mRNA-expressing cells in the control group was demonstrated. This study showed significant improvement in symptoms, medication use, and immunologic parameters suggesting benefit for at least 3 years after completion of a 3-4 year course of immunotherapy treatment.
LONG-TERM CLINICAL EFFICACY OF GRASS-POLLEN IMMUNOTHERAPY

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ABSTRACT

Background  Pollen immunotherapy is effective in selected patients with IgE-mediated seasonal allergic rhinitis, although it is questionable whether there is long-term benefit after the discontinuation of treatment.

Methods  We conducted a randomized, double-blind, placebo-controlled trial of the discontinuation of immunotherapy for grass-pollen allergy in patients in whom three to four years of this treatment had previously been shown to be effective. During the three years of this trial, primary outcome measures were scores for seasonal symptoms and the use of rescue medication. Objective measures included the immediate conjunctival response and the immediate and late skin responses to allergen challenge. Cutaneous-biopsy specimens obtained 24 hours after intradermal allergen challenge were examined for T-cell infiltration and the presence of cytokine-producing T helper cells (T\(_2\) cells) (as evidenced by the presence of interleukin-4 messenger RNA). A matched group of patients with hay fever who had not received immunotherapy was followed as a control for the natural course of the disease.

Results  Scores for seasonal symptoms and the use of rescue antiallergic medication, which included short courses of prednisolone, remained low after the discontinuation of immunotherapy, and there was no significant difference between patients who continued immunotherapy and those who discontinued it. Symptom scores in both treatment groups (median areas under the curve in 1995, 921 for continuation of immunotherapy and 504 for discontinuation of immunotherapy; P = 0.60) were markedly lower than those in the group that had not received immunotherapy (median value in 1995, 2863). Although there was a tendency for immediate sensitivity to allergen to return late after discontinuation, there was a sustained reduction in the late skin response and associated CD3+ T-cell infiltration and interleukin-4 messenger RNA expression.

Conclusions  Immunotherapy for grass-pollen allergy for three to four years induces prolonged clinical remission accompanied by a persistent alteration in immunologic reactivity. (N Engl J Med 1999; 341:468-75.)

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Despite advances in pharmacotherapy for grass-pollen allergy, there has been a marked increase in the prevalence of summer hay fever in countries with a Western lifestyle. Although topical nasal corticosteroids and the new nonsedating antihistamines are highly effective in treating hay fever, there remains a group of patients who have a poor response to these treatments and for whom immunotherapy is currently recommended. An important question is whether allergen immunotherapy exerts a prolonged effect after it is discontinued. Such an effect would make this form of therapy attractive for prophylaxis and for early intervention.

We previously demonstrated the usefulness of immunotherapy in a cohort of patients with severe summer hay fever that could not be controlled by antiallergic drugs. We initially followed these patients for a four-year period. During the first year (1989), patients were randomly assigned to receive injections of either grass-pollen vaccine or placebo. The vaccine was highly effective in reducing symptoms and the need for rescue drugs. Efficacy was accompanied by decreased sensitivity of the conjunctiva and skin to allergen and by inhibition of the late skin response. Clinical improvement was maintained with continued immunotherapy during the ensuing three years.

In the current, placebo-controlled study, we examined the effects of the discontinuation of immunotherapy for three years in the same group of patients. We also followed a matched group of patients who never received immunotherapy as a control for the natural history of the disease during this phase. Objective measures of outcome included immediate sensitivity of the conjunctiva and early and late skin responses to grass-pollen extract. Immunologic responsiveness was determined by assessing the late infiltration of CD3+ T lymphocytes and production of interleukin-4 in skin specimens 24 hours after intradermal grass-pollen challenge. We tested the hy-
LONG-TERM CLINICAL EFFICACY OF GRASS-POLLEN IMMUNOTHERAPY

METHODS

Patients

In 1988, 40 patients were recruited from the Royal Brompton Hospital Allergy Clinic or through an advertisement in a local newspaper; these patients had a history of severe seasonal allergic rhinitis, poor control of symptoms in previous years despite regular use of antiallergic drugs, and positive skin-prick test (wheal > 5 mm) to timothy grass-pollen extract. Patients were excluded if they had a clinical history of other allergies or important medical illnesses or if they had chronic asthma. Patients with mild seasonal asthma were included, provided their symptoms were controlled by inhaled sympathomimetic β₂-adrenergic–agonist bronchodilators. Thirty-seven patients completed the initial one-year, double-blind, placebo-controlled study (1989). For patients who received placebo injections during that year, immunotherapy with grass-pollen extract was then initiated over a six-to-eight-week period, and subsequently 32 of the 37 patients completed maintenance therapy with grass-pollen injections for the following three years (1990 through 1992). In 1992, 15 matched patients with hay fever who had never received immunotherapy were recruited as a control group; the inclusion and exclusion criteria were identical to those used for the patients who received immunotherapy.

Study Design

The study was performed with the approval of the Royal Brompton Hospital ethics committee, and all the patients gave written informed consent. In 1992, 32 patients remained in the group receiving immunotherapy; analyses of data on these patients were stratified according to whether they had received three or four years of active immunotherapy before the current, double-blind randomization to either continued maintenance immunotherapy with depot grass-pollen vaccine (the maintenance group) or matched placebo injections (the discontinuation group). Injections of vaccine or placebo were given monthly for three years. The 15 matched patients who had never received immunotherapy (the control group) received no injections and were monitored in parallel. Patients in all three groups had equal access to the same rescue medication and underwent the same follow-up assessments.

Immunotherapy

A standardized, aluminum hydroxide–adsorbed, depot grass-pollen vaccine (Alutard SQ, ALK Abelló, Horsholm, Denmark) was used for subcutaneous immunotherapy. Each monthly 1-ml maintenance injection contained 100,000 SQ units (equivalent to 10,000 biologic units*) and containing 20 µg of the phleum [timothy] allergen P5. Placebo injections consisted of identical vials of diluent, including aluminum hydroxide and 0.01 mg of histamine per milliliter. For three years, 1-ml injections were given monthly in the upper arm, except during the pollen seasons, when the maintenance dose was reduced by 40 percent. Patients were observed for one hour after each injection.

Assessments

Primary outcome measures were the presence of symptoms and the need for rescue medication. Patients recorded symptom scores and drug requirements every day from May through September of each year. Individual symptoms in the nose (sneezing, blockage, and running), eyes (itching, redness, tears, and swelling), mouth and throat (itching and dryness), and chest (breathlessness, cough, wheezing, and tightness) were recorded on a scale of 0 to 3 (with a score of 0 indicating no symptoms and 1, 2, and 3 indicating mild, moderate, and severe symptoms, respectively). Patients were given cromolyn sodium eye drops (Opticrom, Rhone-Poulenc, West Malling, United Kingdom), aqueous nasal spray (cromolyn sodium, Rynacrom, Laboratoires Fisons, Le Trait, France), a short-acting, nonsedating antihistamine, acrivastine (8-mg capsules, Sepremper, Glaxo Wellcome, Greenford, United Kingdom), and an albuterol inhaler (Ventolin, Allen and Hanburys, Stockley Park, United Kingdom) as rescue medications. If symptoms were not controlled, patients were advised to take, in addition, a seven-day course of prednisolone tablets (5-mg tablets; dosage, 30 mg per day for two days, with the dose successively reduced by 5 mg on each of the following five days).

Patients’ diaries were scored by totaling individual symptom scores for each week, with a maximal possible score of 21 for each symptom. Drugs were scored as follows: each eye drop, dose of nasal spray, or inhalation of albuterol was given a score of 1, and each acrivastine capsule or prednisolone tablet was given a score of 2. Patients were asked every two weeks to record a visual-analogue score (on a scale of 0 to 10, where 0 indicated minimal symptoms and 10 indicated maximal symptoms) in response to the question, “How has your hay fever been during the past week?”

Objective measures of response and control of symptoms in each group receiving immunotherapy and the immediate and late skin responses and the immediate conjunctival response to allergen challenge. They were assessed at the end of the study in November 1995. Skin-prick tests were performed in duplicate, with allergen concentrations of 100 to 100,000 SQ units per milliliter applied to the flexor aspect of the forearm. Immediate responses were recorded after 15 minutes and were expressed as the allergen concentration that caused a 6-mm wheal. Intradermal testing was performed on the extensor surface of the forearm with an injection of 10 SQ units of allergen in 0.02 ml of diluent and a control injection of diluent alone. Late responses were recorded as the mean diameter of the swelling at 24 hours.

Tests of the conjunctival response were performed by instilling half-log (approximately threefold) incremental concentrations of grass-pollen extract (100 to 100,000 SQ units per milliliter) into alternate eyes at 10-minute intervals. Immediate conjunctival sensitivity was recorded as the dose that induced a minimum of two of four symptoms (itching, redness, tears, or swelling). In both the skin and conjunctival tests, if there was no response at the highest concentration of allergen tested (100,000 SQ units per milliliter), the outcome was arbitrarily assigned a value of 300,000 SQ units per milliliter.

Skin Biopsies

Punch-biopsy specimens 3 mm in diameter were taken 24 hours after intradermal injection from both the site of allergen injection and the site of diluent (control) injection. CD3+ T cells and cells containing interleukin-4 messenger RNA (mRNA) were identified by immunohistochemical analysis and in situ hybridization of 6-µm cryostat sections of the biopsy specimens with appropriate negative controls, as previously described. All analyses were performed in a blinded manner.

Statistical Analysis

Symptom and medication scores were expressed as the area under the curve for the 11-week period that corresponded to the peak pollen season. The primary outcome was analyzed by comparing symptom and rescue-medication scores over the three-year period of the study for the maintenance group with scores for the discontinuation group. Visual-analogue scores during the pollen season, the results of skin and conjunctival tests, and cell counts in skin-biopsy sections were analyzed in the same way with use of the two-tailed Mann–Whitney U tests. P values of less than 0.05 were considered to indicate statistical significance.

RESULTS

The three groups of patients were matched for sex and age. They were also matched for wheal size in...
Diameter of wheal on skin-prick testing at enrollment (1988 for both immunotherapy groups and 1992 for the control group) (Table 1). Throughout the current trial, weekly pollen counts in London peaked consistently in the average or above-average range (60 ±24 SE grains per cubic meter for the years 1989 to 1998) in June and July (Fig. 1). Scores for total hay fever symptoms, rescue medication, and the visual-analogue scale for both the maintenance group and the discontinuation group were temporally related to pollen counts, remained low, and were similar to those recorded during the preceding three years, when all the patients in these two groups had received active immunotherapy.5 There were no significant differences in any of these scores between these two groups throughout the three-year period. Individual nose, eye, chest, mouth, and throat symptoms also remained similar between these two groups (Table 2). In contrast, symptom and rescue-medication scores in both groups were markedly lower than those in patients in the control group (Fig. 1 and Table 2). The need for one or more courses of prednisolone tablets during the three-year period was also markedly lower in the maintenance and discontinuation groups (3 of 16 patients in each group) than in the control group (9 of 15 patients).

There was a tendency for immediate sensitivity to grass pollen to return three years after the discontinuation of immunotherapy. The concentration of grass-pollen extract required to cause a 6-mm wheal on skin-prick testing was significantly lower in the group that discontinued immunotherapy (median, 40,000 SQ units per milliliter; range, 3000 to 300,000) than in the group that received maintenance immunotherapy (median, 300,000 SQ units per milliliter; range, 60,000 to 300,000; P = 0.005). There was also a trend in the discontinuation group toward a decrease in the grass-pollen concentration that elicited an immediate conjunctival response (median, 30,000 SQ units per milliliter; range, 300 to 300,000, as compared with 100,000 SQ units per milliliter; range, 3000 to 300,000, in the maintenance group; P = 0.06). In the control group, however, the concentrations of allergen that caused an immediate wheal (median, 3000 SQ units per milliliter; range, 1000 to 10,000) and immediate conjunctival symptoms (median, 3000 SQ units per milliliter; range, 300 to 30,000) remained markedly lower than those in both immunotherapy groups (maintenance and withdrawal).

The control group had large (>3 cm) late skin responses 6 to 48 hours after the intradermal injection of grass pollen (Fig. 2). Immunohistochemical analyses of biopsy specimens from the site of allergen injection, as compared with control sites, revealed marked infiltration at 24 hours by CD3+ T lymphocytes and an increase in cells containing interleukin-4 mRNA. In contrast, in both the maintenance group and the discontinuation group, the late skin response was virtually absent and there were fewer infiltrating CD3+ T cells and markedly fewer infiltrating cells containing interleukin-4 mRNA than in the control group (Fig. 2). No differences were observed between these two groups in the late skin response as assessed on the basis of the size of the swelling (P = 0.16) or in the numbers of CD3+ T cells (P = 0.57) or cells containing interleukin-4 mRNA (P = 0.87). In both immunotherapy groups, there was no correlation between the late skin responses and the corresponding immediate skin responses after intradermal injection of grass pollen (data not shown).

Immunotherapy was well tolerated by the patients who received it throughout the three-year period. Less than 2 percent of injections resulted in early or delayed local reactions larger than 3 cm in diameter. No substantial immediate or late systemic reactions were observed after allergen injections. Thirty-nine of the 47 patients completed the study. During the second and third years of the study, 2 of the 16 patients in the maintenance group, 3 of the 16 in the discontinuation group, and 3 of the 15 in the control group dropped out. These patients withdrew for reasons unrelated to the study protocol. Blinding of the trial was checked at the end of the study by asking the patients and the investigator to guess which treatments (active [maintenance] or placebo [discontinuation]) the patients in the two immunotherapy groups had received. Sixteen of the 27 patients remaining in these two groups guessed correctly, whereas the investigator was correct in 15 of the 27 cases. These results were not significantly different from those that would have occurred by chance (P = 0.5 for the patients’ guesses, and P = 0.8 for the investigator’s guesses, by the chi-square test).

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**Table 1. Clinical Characteristics of the 47 Patients Studied from 1992 Through 1995.**

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>IMMUNOTHERAPY 1989–1992</th>
<th>NO IMMUNOTHERAPY (N=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAINTENANCE (N=16)</td>
<td>DISCONTINUATION (N=16)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>11/5</td>
<td>8/8</td>
</tr>
<tr>
<td>Age in 1992 (yr)</td>
<td>38</td>
<td>42</td>
</tr>
<tr>
<td>Median</td>
<td>32–48</td>
<td>33–50</td>
</tr>
<tr>
<td>Diameter of wheal on skin-prick testing (mm)*</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>8–12</td>
<td>9–14</td>
</tr>
</tbody>
</table>

*Values represent the size of the wheal in response to skin-prick testing with timothy allergen at the time of enrollment.
DISCUSSION

We have shown, under double-blind conditions, that three to four years of grass-pollen immunotherapy remains effective for at least three years after the discontinuation of the injections. In both the group that received maintenance immunotherapy and the group that discontinued immunotherapy, clinical improvement was accompanied by a marked decrease in the late skin response to allergen challenge, blunting of the accompanying CD3+ T-cell infiltrate, and fewer interleukin-4 mRNA–expressing cells than in the control group. The results demonstrate prolonged clinical benefit and provide evidence of decreased immunologic reactivity for at least three years after the discontinuation of immunotherapy for the treatment of hay fever.

In contrast to the late skin response, there was a tendency for immediate sensitivity to grass pollen to return three years after discontinuation of immunotherapy, as reflected by the appearance of a small but significant difference in the immediate response to skin-prick testing and a trend toward an increase in conjunctival sensitivity (measured 15 minutes after allergen challenge) in the discontinuation group as compared with the maintenance group. However, this tendency was not accompanied by a return of symptoms, perhaps indicating that late responses have greater relevance than early responses to the clinical expression of hay fever.

The efficacy of grass-pollen immunotherapy in patients with seasonal hay fever has been confirmed in many controlled trials. Immunotherapy is also effective, although less so, in patients with seasonal asthma. In contrast, patients with perennial disease associated with sensitivity to multiple allergens are less responsive to this form of treatment.

<table>
<thead>
<tr>
<th>OUTCOME MEASURE</th>
<th>IMMUNOTHERAPY</th>
<th>POINT ESTIMATE*</th>
<th>P VALUE†</th>
<th>NO IMMUNOTHERAPY</th>
<th>(N=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAINTENANCE</td>
<td>DISCONTINUATION</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GROUP (N=16)</td>
<td>GROUP (N=16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>median (range)</td>
<td>value (95% CI)</td>
<td></td>
<td>median (range)</td>
<td></td>
</tr>
<tr>
<td>Symptoms‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nose</td>
<td>1993</td>
<td>392 (0–1794)</td>
<td>–74 (–325 to 266)</td>
<td>0.65</td>
<td>1389 (469–3346)</td>
</tr>
<tr>
<td></td>
<td>1994</td>
<td>453 (0–2107)</td>
<td>67 (–287 to 490)</td>
<td>0.68</td>
<td>1358 (165–3360)</td>
</tr>
<tr>
<td></td>
<td>1995</td>
<td>679 (0–1274)</td>
<td>–5 (–462 to 462)</td>
<td>0.98</td>
<td>1225 (241–3066)</td>
</tr>
<tr>
<td>Eyes</td>
<td>1993</td>
<td>140 (0–1554)</td>
<td>–42 (–301 to 98)</td>
<td>0.52</td>
<td>959 (42–3024)</td>
</tr>
<tr>
<td></td>
<td>1994</td>
<td>119 (0–1526)</td>
<td>7 (–266 to 290)</td>
<td>0.81</td>
<td>1054 (0–4984)</td>
</tr>
<tr>
<td></td>
<td>1995</td>
<td>82 (0–1015)</td>
<td>21 (–164 to 308)</td>
<td>0.55</td>
<td>1099 (77–4077)</td>
</tr>
<tr>
<td>Chest</td>
<td>1993</td>
<td>0 (0–861)</td>
<td>0 (0 to 14)</td>
<td>0.60</td>
<td>290 (0–3517)</td>
</tr>
<tr>
<td></td>
<td>1994</td>
<td>0 (0–1365)</td>
<td>0 (0 to 49)</td>
<td>0.42</td>
<td>262 (0–3766)</td>
</tr>
<tr>
<td></td>
<td>1995</td>
<td>0 (0–749)</td>
<td>0 (0 to 28)</td>
<td>0.30</td>
<td>84 (0–3353)</td>
</tr>
<tr>
<td>Mouth and throat</td>
<td>1993</td>
<td>35 (0–357)</td>
<td>0 (–56 to 133)</td>
<td>0.58</td>
<td>301 (0–1491)</td>
</tr>
<tr>
<td></td>
<td>1994</td>
<td>35 (0–564)</td>
<td>7 (–70 to 199)</td>
<td>0.47</td>
<td>416 (0–1841)</td>
</tr>
<tr>
<td></td>
<td>1995</td>
<td>116 (0–865)</td>
<td>0 (–217 to 112)</td>
<td>0.94</td>
<td>452 (0–1690)</td>
</tr>
<tr>
<td>Total</td>
<td>1993</td>
<td>626 (70–3528)</td>
<td>–70 (–613 to 634)</td>
<td>0.85</td>
<td>2615 (609–10,416)</td>
</tr>
<tr>
<td></td>
<td>1994</td>
<td>779 (0–5030)</td>
<td>229 (–487 to 1141)</td>
<td>0.53</td>
<td>3220 (1106–13,951)</td>
</tr>
<tr>
<td></td>
<td>1995</td>
<td>921 (0–2299)</td>
<td>133 (–690 to 1656)</td>
<td>0.60</td>
<td>2863 (774–12,033)</td>
</tr>
<tr>
<td>Rescue-medication use‡</td>
<td>1993</td>
<td>756 (0–4553)</td>
<td>54 (–724 to 2009)</td>
<td>0.85</td>
<td>3672 (1029–10,832)</td>
</tr>
<tr>
<td></td>
<td>1994</td>
<td>1134 (0–5820)</td>
<td>4 (–1064 to 2121)</td>
<td>0.96</td>
<td>4088 (1141–10,808)</td>
</tr>
<tr>
<td></td>
<td>1995</td>
<td>672 (0–1827)</td>
<td>11 (–689 to 1488)</td>
<td>0.88</td>
<td>4729 (1197–8505)</td>
</tr>
<tr>
<td>Visual-analogue scale§</td>
<td>1993</td>
<td>2.9 (0.2–8.2)</td>
<td>–1 (–2.6 to 0.3)</td>
<td>0.13</td>
<td>6.0 (0.7–8.1)</td>
</tr>
<tr>
<td></td>
<td>1994</td>
<td>4.4 (0–8.0)</td>
<td>0 (–3 to 3.1)</td>
<td>0.92</td>
<td>7.8 (1.1–9.0)</td>
</tr>
<tr>
<td></td>
<td>1995</td>
<td>1.9 (0–8.5)</td>
<td>0.2 (–1.9 to 1.6)</td>
<td>0.87</td>
<td>6.4 (0–8.6)</td>
</tr>
</tbody>
</table>

*The point estimates and 95 percent confidence intervals (CIs) were calculated with Minitab software.†P values are for the comparison between the maintenance group and the discontinuation group.
‡Data shown are the medians and ranges for the area under the curve (the scores) during the 11-week pollen season.
§Data shown are median scores for a one-week period during the peak of the pollen season.

Previous studies have suggested that immunotherapy has a long-term effect.20–25 In a retrospective study of children who were sensitive to house-dust mites, short-term (12-month) immunotherapy was associated with a greater rate of relapse than was treatment for more than 3 years. In the only blinded study of the discontinuation of pollen immunotherapy, patients with sensitivity to ragweed were followed for one year; in a finding consistent with our own, a recurrence of immediate sensitivity to allergen was observed.26

T-cell–derived cytokines play a key part in allergic inflammation. Grass-pollen–specific T cells from patients with atopy produce greater quantities of cytokines such as interleukin-4, interleukin-13, and interleukin-5 (and thus can be identified as type 2 T cells)27,28 than do cells from control subjects without atopy, which favor the production of interferon-γ (T(H)1 cells).27,29 Interleukin-430 and interleukin-1331 stimulate IgE production by B cells and therefore promote the sensitization of high-affinity IgE receptors on the surface of mast cells and basophils, whereas interleukin-5 has specific procosinophilic properties.32 IgE-dependent activation of mast cells results in an immediate response to allergen and may contribute to the development of the late response.33

Previous studies found decreases in serum IgE concentrations,34 increases in IgG,35 and inhibition of recruitment or activation of effector cells such as mast cells36,37 and eosinophils38,39 in the target organ in response to immunotherapy. Since each of these processes is thought to be largely T-cell–dependent, one possibility is that immunotherapy exerts a prolonged effect by altering the T-cell response to subsequent allergen exposure. Our earlier studies, performed in the same group of patients after they had received one year of immunotherapy, demonstrated inhibition of the late response in both nose and skin, accompanied by an increase in T(H)1 responses.
as detected by an increase in interferon-γ mRNA expression.\textsuperscript{7,40}

Further studies of cutaneous-biopsy specimens obtained at 24 hours suggested that this Th1 response may have been driven by interleukin-12, since inhibition of the late skin response was accompanied by a marked increase in cells expressing interleukin-12 mRNA, predominantly tissue macrophages.\textsuperscript{41} Studies of T-cell responses in the peripheral blood of patients undergoing immunotherapy with pollen extract have revealed a corresponding reduction in Th2 responses, as shown by a decrease in interleukin-4.\textsuperscript{42,43} Taken together, these studies suggest that pollen immunotherapy may act either by inducing immune deviation of Th1,2 and Th0 T-cell responses in favor of Th1 responses or by diminishing Th2 and Th0 T-cell responses.\textsuperscript{44}

In contrast to our earlier findings, the current finding of a decrease in the number of cells expressing interleukin-4 mRNA suggests that persistent suppression of Th2 responses may be responsible for sustained clinical improvement, as reflected by an inhibition of the late response, whereas immediate mast-cell–dependent responses to allergen may return several years after discontinuation of treatment. Since we did not identify the cellular source of interleukin-4 mRNA, it is possible that cells other than T cells, including basophils,\textsuperscript{45} mast cells,\textsuperscript{46} or eosinophils,\textsuperscript{47} contribute to the expression of this cytokine. Irrespective of the precise mechanism, these data provide objective evidence of a long-term immunologic effect after the discontinuation of immunotherapy.

The usefulness of allergen immunotherapy is highlighted in a recent World Health Organization report,\textsuperscript{3} which advocates its use in selected patients with specific IgE antibodies to clinically relevant allergens. Selection of patients is extremely important; the risk–benefit ratio is less favorable for patients with asthma than for those with allergic rhinitis. The rationale for prescribing allergen immunotherapy depends on the degree to which symptoms can be alleviated by medication and whether effective avoidance of allergen is possible. The quality of allergen vaccines is also critical, and an optimal maintenance dose of 5 to 20 µg of major allergen per injection (as in the current study) correlates with clinical efficacy.\textsuperscript{3}

An important question has been whether immunotherapy has the potential to modify the long-term course of allergic disease after discontinuation.\textsuperscript{19–25} The current findings suggest that it does and raise the question of whether allergen-injection immunotherapy should be considered earlier in the course of allergic disease to prevent progression or, as suggested by another study,\textsuperscript{48} the development of multiple allergies. Further studies with long-term follow-up, particularly in children with limited allergic sensitivities, could address this possibility.

Figure 2. Late Skin Responses and Number of CD3+ T Cells and Cells Containing Interleukin-4 mRNA in Skin-Biopsy Specimens Obtained 24 Hours after Intradermal Injection of Allergen or Diluent.

Late skin responses were assessed by intradermal injection of either grass-pollen extract or the diluent alone as a negative control at the end of the study. Late skin responses are expressed as the size of the swelling as measured 24 hours after intradermal allergen challenge. Twelve patients in the maintenance-therapy group, 13 in the group that discontinued therapy, and 11 in the control group underwent skin biopsy. Cell counts are expressed per square millimeter of skin. Values for individual patients (circles) and median values (squares) are shown. Data on interleukin-4 mRNA were not obtained for one patient in the maintenance-therapy group, two in the group that discontinued therapy, and two in the control group because the tissue architecture of their biopsy specimens was distorted during processing for in situ hybridization.

