TWELVE-YEAR FOLLOW-UP AFTER DISCONTINUATION OF PRESEASONAL GRASS POLLEN IMMUNOTHERAPY IN CHILDHOOD

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A small study that tracked twenty-two patients 12 years after specific immunotherapy (SIT) treatment course of 3 years was discontinued. The primary end points for this study included symptom score, medication use, and combined symptom and medication score. Skin test reactivity to the development of new sensitizations and prevalence for seasonal asthma were also evaluated. Patients were selected and matched for age, gender, prevalence of seasonal asthma and wheal size in response to skin prick test with grass pollen during study enrollment. A reduction in symptom scores ($P < 0.03$), medication use ($P < 0.05$), combined symptom and medication score ($P < 0.03$) remained low in comparison to the control group. Even though there was immediate skin reactivity to allergen at the twelve year point, it was not accompanied with symptoms. Sensitization to new allergens remained low (58%), compared to control group (100%. $P < 0.05$), with most new sensitizations identified as cat, dog, and house dust mites. Seasonal asthma also tended to be lower in the post-SIT group as well ($P = 0.08$).
Original article

Twelve-year follow-up after discontinuation of preseasonal grass pollen immunotherapy in childhood

Background: In a previous controlled study, we demonstrated that preseasonal grass pollen immunotherapy for 3 years was effective in children. Moreover, a significant clinical benefit could still be observed 6 years after discontinuation of specific immunotherapy (SIT). In the current study, we examined the same group of patients again to investigate whether there is a prolonged benefit 12 years after SIT is stopped.

Methods: Twenty-two patients with previous SIT (from 1989 through 1991) or standardized seasonal pharmacotherapy only were prospectively followed during the grass pollen season of 2003. Primary end points were symptom score, medication use, and combined symptom and medication score. In addition, skin prick test reactivity, development of new sensitizations, and prevalence of seasonal asthma were evaluated.

Results: Total hay fever symptom score ($P < 0.03$), use of medication ($P < 0.05$), and combined symptom and medication score ($P < 0.03$) remained lower in patients with previous SIT when compared with the control group. Decreased immediate skin response to grass pollen returned 12 years after cessation of SIT. The percentage of new sensitization, however, continued to be significantly smaller in patients with previous SIT (58%) compared with the controls (100%, $P < 0.05$). There was a tendency for lower prevalence of seasonal asthma in the post-SIT group ($P = 0.08$).

Conclusion: This prospective controlled prolonged follow-up study demonstrates the ongoing clinical benefit 12 years after discontinuation of SIT. Furthermore, the reduction in onset of new sensitization, which was found 6 years after discontinuation of SIT, is sustained 6 years later.

New data demonstrate the efficacy of specific immunotherapy (SIT) not only as a therapeutic agent but also as a preventive strategy to reduce onset of new sensitization to nonrelated allergens (1−3), progression from allergic rhinitis to asthma (4, 5), and to improve long-term outcome of already established asthma (3, 6).

However, only limited knowledge exists about the duration of the preventive and therapeutic effects after discontinuation of SIT. The main objectives of this study were to evaluate whether grass pollen SIT in childhood is still effective 12 years after discontinuation and to test whether the reduced onset of new sensitization is prolonged.

Material and methods

Patients and study design

The study population has been described previously (3, 7). Briefly, in 1988 we recruited 28 children with a history of severe grass pollen allergic rhinoconjunctivitis for at least 2 years with or without seasonal asthma but with immunoglobulin (IgE)-mediated sensitivity to seasonal allergens only (grass pollen with or without tree pollen). Subjects with a history of other allergic disease or sensitization to nonpollen allergens were excluded.

The original study was a nonrandomized controlled open trial. The SIT was proposed to all patients fulfilling the inclusion criteria, but some children and/or their parents declined to receive SIT. These patients were included as controls (3, 7). From 1989 through 1991 patients were either treated with grass pollen depot-allergoids (Allergovit®; Allergopharma, Rheinbeck, Germany) in a preseasonal immunotherapy protocol ($n = 14$) or received standardized pharmacotherapy alone during the grass pollen season ($n = 14$). A first 6-year follow-up study was performed during the grass pollen season of 1997 (3). The present study is a second follow-up study 12 years after discontinuation of SIT, designed as a prospective controlled open study during the grass pollen season of 2003. Except for one subject who could no longer be traced, all patients of the previous follow-up study could be recruited.

Assessments

Primary end points were the presence of symptoms and the need for medication from May 1 until July 31. Symptom scores, medication scores, and combined symptom and medication scores
were calculated exactly as detailed before (3). Secondary end points included skin prick test (SPT) reactivity and allergen-specific IgE to grass pollen (3), assessment of newly developed sensitization by SPT using the same panel of allergens as at study enrollment and at the first follow up (3, 7), and prevalence of seasonal asthma defined as occurrence of at least two of the following symptoms during the grass pollen season: cough, wheeze, dyspnea, and exercise intolerance. In contrast to our previous follow-up study, conjunctival provocation tests were not performed. A substantial number of patients declined to undergo the provocation test resulting in too low numbers of subjects for statistical analysis.

Statistics

The two-tailed Mann–Whitney U-test (Statview Software, Cary, NC, USA) was used for comparison between groups regarding symptom and medication scores. Assessment of seasonal asthma and occurrence of new sensitization were compared by means of chi-squared test. A 5% significance level was used.

Results

The two study groups, observed over a total length of 15 years, were matched for gender, age, prevalence of seasonal asthma, and wheal size in response to SPT with grass pollen at study enrolment (Table 1).

Clinical efficacy

Scores for hay fever symptoms \((P < 0.03)\), for use of medication \((P < 0.05)\) and combined symptom and medication score \((P < 0.03)\) expressed as area under the curve for the grass pollen season remained significantly lower in patients 12 years after cessation of SIT compared with the controls. The scores were temporally related to pollen counts (Fig. 1).

Table 2 compares the data of the current 12-year follow up with those of the 6-year follow-up study. In 2003, grass pollen release began at the end of April already and peaked for several consecutive weeks in May and June resulting in an increased number of days with high grass pollen concentration compared with 1997.

The difference in symptom scores between the two groups was smaller in 2003 when compared with 1997, but still reaching statistical significance. However, in contrast to 1997, control patients used significantly more medication for symptom relief in 2003 than patients in the post-SIT group. Symptom plus medication score remained markedly lower in patients with previous SIT than in controls.

Table 1. Characteristics of patients studied from 1988 to 2003

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Number of patients recruited during 1988</td>
<td>14</td>
</tr>
<tr>
<td>Median age (years) and range</td>
<td>9.5 (5–16)</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>10/4</td>
</tr>
<tr>
<td>Result of skin prick testing at study enrolment*</td>
<td>2.49</td>
</tr>
<tr>
<td>Number of patients at first follow up (1997)</td>
<td>13</td>
</tr>
<tr>
<td>Number of patients at second follow up (2003)</td>
<td>12</td>
</tr>
<tr>
<td>Median age (years) and range</td>
<td>23.8 (20–31)</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>9/3</td>
</tr>
</tbody>
</table>

*Values represent the quotient of mean wheal area to grass and mean wheal area of histamine control.

Figure 1. Median weekly pollen count, total hay fever symptom score, and combined symptom plus medication score during the pollen season 2003. *\(P\)-values are for the comparison between the two groups (analysis of the area under the curve). (A) Median weekly grass pollen count; (B) hay fever symptom score; (C) symptom and medication score.
Skin prick tests and specific IgE

The SPT reactivity to grass pollen allergens, which was significantly decreased after discontinuation of SIT in 1991 (7) and at the 6-year follow up in 1997 (3), returned to the magnitude of the controls in the present study (Fig. 2A). There were no differences in the amount of IgE specific for grass pollen between the groups at enrolment (7), at commencement and discontinuation of SIT (7) as well as at the follow-up studies in 1997 and 2003 (data not shown).

Evolution of sensitizations and asthma prevalence

Six years after discontinuation of SIT, a significantly reduced number of patients had developed new sensitizations when compared with the controls (3). This reduction in development of new sensitizations after SIT was confirmed in the current 12-year follow-up study (P < 0.05; Fig. 2B). Most new sensitizations occurred to house dust mites, cat, and dog dander. In addition to grass pollen, 40% of the controls and 42% of the actively treated group were sensitized to tree pollen at study enrolment. At the 12-year follow up, the prevalence of sensitization to tree pollen was 90% in the controls and 67% in the post-SIT group.

After discontinuation of SIT, prevalence of seasonal asthma because of grass pollen decreased significantly (7). This reduction was sustained in 1997 (3). In the 12-year follow up there was still a tendency for lower asthma prevalence in the post-SIT group (Fig. 2C), but without reaching statistical significance (P = 0.08).

Discussion

The present study assesses two groups of patients 15 years after enrolment for grass pollen SIT or standardized seasonal pharmacotherapy alone. To our knowledge it is the longest follow-up study of grass pollen SIT. The data demonstrate an ongoing clinical benefit 12 years after cessation of 3-year preseasonal SIT with grass pollen allergoids in childhood in terms of both a reduction of hay fever symptoms and use of medication for symptom relief. Furthermore, the reduction in development of new sensitizations to perennial allergens, which has been observed 6 years after cessation of SIT (3), is sustained also in the longer term.

Only a few papers have addressed the long-term effects of grass pollen SIT (3, 5, 8–12). Most studies demonstrate prolonged clinical benefit and some show decreased

Table 2. Pollen concentration, median scores for total hay fever symptoms, and symptom plus medication scores during the pollen seasons 1997 and 2003

<table>
<thead>
<tr>
<th>1997</th>
<th>2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of days with high-grass pollen concentration (&gt;50 grains/m³/24 h)</td>
<td>32</td>
</tr>
<tr>
<td>Median total symptom score (range)</td>
<td>45.0 (0–154)</td>
</tr>
<tr>
<td>No SIT</td>
<td>104.0 (42–344)</td>
</tr>
<tr>
<td>SIT</td>
<td>P-value*</td>
</tr>
<tr>
<td>Median medication score (range)</td>
<td>10.0 (0–141)</td>
</tr>
<tr>
<td>No SIT</td>
<td>30.5 (0–138)</td>
</tr>
<tr>
<td>SIT</td>
<td>P-value*</td>
</tr>
<tr>
<td>Median symptom plus medication score (range)</td>
<td>66.0 (0–278)</td>
</tr>
<tr>
<td>No SIT</td>
<td>146.0 (44–764)</td>
</tr>
<tr>
<td>SIT</td>
<td>P-value*</td>
</tr>
</tbody>
</table>

*P-values are for the comparison between the two groups in 1997 and 2003. Analysis of the area under the curve was performed using the Mann–Whitney U-test.
immunologic reactivity for 3–6 years after discontinuation of SIT. The majority has been performed in adults (5, 8–12) and some of them were not controlled (5, 8). There is evidence that immunologic reactivity begins to return after 1–3 years (8, 10, 12). In our patients, recurrence of immediate skin reactivity to allergen occurred later than 6 years after cessation of SIT. However, increased reactivity to grass pollen was not accompanied by an increase of symptoms.

Changes in immunologic parameters and provocation tests may be of interest in elucidating mechanisms, but cannot replace clinical evaluation (13). The only parameter estimating clinical efficacy of SIT are reduction in symptoms and use of medication. However, individual patient behavior during the pollen season may be different. Some are using more medication for rapid symptom relief. Others may have decreased symptom perception and subjectively underestimate symptom severity. The addition of weekly combined symptom and medication scores may partly compensate for individual patient behavior. However, a shortcoming of all immunotherapy studies remains that there is currently no objective means to monitor clinical efficacy of SIT.

An important finding of recent studies is the reduction in onset of new sensitization to nonrelated allergens after SIT. Our data are in accordance with the results of two objective means to monitor clinical efficacy of SIT. The majority has been performed in adults (5, 8–12) and some of them were not controlled (5, 8). There is evidence that immunologic reactivity begins to return after 1–3 years (8, 10, 12). In our patients, recurrence of immediate skin reactivity to allergen occurred later than 6 years after cessation of SIT. However, increased reactivity to grass pollen was not accompanied by an increase of symptoms.

In conclusion, this prospective controlled long-term follow-up study demonstrates that both the clinical efficacy and the preventive capacity of grass pollen SIT, observed at the 6-year follow up, is still evident 12 years after discontinuation of SIT when compared with seasonal pharmacotherapy alone.

References

Allergen immunotherapy (IT) alters the course of allergic diseases through a series of repeated subcutaneous injections or sublingual doses of extracts composed of clinically relevant allergens. This review consisted of a broad search of articles on immunotherapy in children. Considered in the review were double-blind, placebo-controlled, randomized clinical trials, randomized open controlled trials, and retrospective and post-marketing studies. Pediatric immunotherapy studies noted same dosage for both adult and pediatric patients (SCIT and SLIT). Clinical efficacy of immunotherapy by Eng et.al (article provided already) noted supporting data of SCIT in the prevention of new sensitizations and asthma onset 7 years post IT and reduction of symptoms 12 years after a 5 year course treatment. SLIT demonstrated clinical efficacy for pollen-induced allergic rhino conjunctivitis and seasonal asthma when used daily versus dosing at lower frequency. Best effect noted if the treatment continued for at least 1 year. Pre-seasonal SLIT dosing at least 4 months before start of pollen season was noted as helpful, though this result was not true in 3 of 8 seasonal studies. Two of four studies for SLIT’s effectiveness in perennial asthma were doubtful which may be due to asthma pathology being multi-factorial.
For this review, articles on immunotherapy dosing in pediatric respiratory allergy were identified via PubMed, through congressional abstracts for 2008, in reference lists of recent review articles, and via personal communication with experts. In pediatric subcutaneous immunotherapy (SCIT), doses shown to be effective, mostly in aluminum-adsorbed preparations administered every 6 weeks, contain 20 μg of group 5 major allergen, 12 μg Bet v 1, 15 μg Fel d 1, and 5 to 20 μg Der p 1. Evidence indicates that SCIT prevents new sensitizations and asthma onset 7 years after discontinuation and reduces symptoms 12 years after a 5-year SCIT course, even though skin reactivity returns. Consistent evidence of effect exists for sublingual immunotherapy in pediatric respiratory allergy with daily 15- to 25-μg grass group 5 major allergen and 6 μg Bet v 1. Der p/1 doses of 0.8/0.4 μg three times weekly (up to 27/57 μg daily) demonstrate inconsistent findings. Evidence of effect exists for SCIT in pediatric allergic rhinitis and asthma as treatment and preventive management. Evidence of effect exists for sublingual immunotherapy in pediatric allergic rhinoconjunctivitis and seasonal asthma. Similar results are doubtful for perennial asthma.

Methods
A broad search was conducted to look for articles on immunotherapy in children—subcutaneous and sublingual—in which the dose of the administered allergen extract was mentioned.

Search strategy
Four search strategies were used to identify articles on allergen immunotherapy in children. For publications on subcutaneous immunotherapy (SCIT), a PubMed search was conducted using the keywords “immunotherapy,” “allergen,” “hyposensitization,” “child,” “children,” and “pediatric” to identify clinical trials published in the past 15 years. For the search for articles on sublingual immunotherapy (SLIT), the comprehensive review on SLIT published by the Joint Task Force on Sublingual Immunotherapy of the American Academy of Allergy, Asthma, and Immunology (AAAAI)/American College of Allergy, Asthma, and Immunology (ACAAI) [6], of
which the author is a member, was taken as a basis reference for articles up to October 2005. To find more recent publications on SLIT, an identical PubMed search was conducted adding the keyword “sublingual.” Second, the search was augmented by scanning references of identical articles and reviews. Third, the most up-to-date information was added by searching the abstracts from the 2008 annual meeting of the AAAAI and from the 2008 annual meeting of the European Academy of Allergy and Clinical Immunology. Finally, some article and abstract authors were asked for more in-depth information, especially on the exact dosing used in the studies.

Types of patients in reviewed articles
In this review, we include original articles in which those recruited were exclusively allergic patients 18 years old or younger with a history of allergic rhinitis, conjunctivitis, and/or asthma in whom the causal allergen was identified and IgE sensitization was ascertained by prick test and/or study of specific IgE assays. Moreover, studies with a mixed pediatric/adult population were added if a pediatric subgroup analysis was included.

Types of intervention
We considered SCIT and SLIT. All appropriate aeroallergens were considered at all doses and all durations of treatment.

Study selection
All types of clinical trials were considered in this review: double-blind, placebo-controlled, randomized clinical trials (DBPCs); randomized, controlled trials (RCTs); open controlled trials; and retrospective and postmarketing studies. These latter studies were only analyzed if the allergen dose was specified.

Results
Dose–response immunotherapy studies in children
Only a few dose–response studies have been published with SCIT, none in the pediatric age group. With SLIT, there is only one published double-blind, placebo-controlled dosing study [7••]. To make any further comments about the dose–effect relationship of immunotherapy in children, individual immunotherapy studies will have to be reviewed, with special attention paid to the doses used and the clinical efficacy reported (Table 1 [SCIT]). However, in most studies published more than a decade ago, the dosing is not mentioned or is expressed in nonuniversal units without reporting the microgram major allergen dose. That is why only some trials from more than 10 years ago are discussed in this review and more attention is paid to more recent studies that express doses.

We first review the efficacy of studies published on SCIT in children and then trials on SLIT in children with allergies. Finally, some trials studying safety are mentioned.

Clinical efficacy of studies for SCIT in children

Double-blind, placebo-controlled trials
Although the DBPC design offers the most valid information on efficacy, only a few trials on SCIT in children were DBPC because of the logical ethical drawbacks. One of the first DBPC trials of SCIT to recruit exclusively pediatric patients dated from the 1980s and investigated SCIT for *Cladosporium* spp [8].

A second DBPC trial [9] of SCIT in asthmatic children (12 active, 11 placebo) who were allergic to *Dermatophagoidea pteronyssinus* showed an improvement in bronchial challenge tests—specific and nonspecific—in both groups. The confounder in this study was that the children were transferred for 12 months to the Italian Alps at the same time. The dose given was expressed in local units without referral to micrograms of major allergen. Another DBPC trial in 121 perennial asthmatic children with a mix of up to seven allergens showed no statistically significant difference between the active and placebo groups, as both improved [10].

Hedlin et al. [11] designed a partially DBPC trial in 29 children with polysensitization to perennial and seasonal allergens. All patients were given pollen immunotherapy, but only the randomized patients (*n* = 15) also received immunotherapy with a perennial allergen: cat or *D. pteronyssinus*. The dose administered at maintenance was 100,000 standard quality units (SQ-U) of a depot extract (Alutard SQ; ALK-Abelló, Horsholm, Denmark) every 6 weeks, corresponding to 15 μg Fel d 1 or 7 μg Der p 1. Specific bronchial hyperreactivity improved in the active group (*P* < 0.001 vs placebo) after 3 years of SCIT. PC20 histamine also improved year by year but did not reach statistical significance in group-to-group comparison. All but one patient in the active group reported experiencing no more symptoms with cat exposure versus none in the placebo group.

A DBPC trial recently was undertaken in China [12] with SCIT for house dust mite in patients with allergic asthma. Of the 132 patients randomized, 85 were 6 to 16 years old, with 44 of them receiving active treatment. The authors found statistically significant improvement of symptoms and medication scores in the SCIT group and a statistically significant reduction in symptoms of verum compared with the placebo group during the maintenance phase of this 1-year trial. However, this improvement was not statistically significant in a subgroup analysis of the pediatric age group. All patients received 100,000 SQ-U (9.8 μg Der p 1 depot preparation [Alutard SQ]) every 6 weeks.

Randomized, controlled trials of SCIT in children

In an RCT of 15 actively treated asthmatic children (14 controls), SCIT with a dialyzed and chemically conjugated *D. pteronyssinus* extract showed efficacy [13]. The investigators demonstrated reduced asthma exacerbations (*P* < 0.01) and reduced use of bronchodilator and systemic corticosteroids (*P* < 0.01) in the active group compared with the control group. Moreover, PC20 methacholine...
### Table 1. Trials with subcutaneous immunotherapy in children

<table>
<thead>
<tr>
<th>Design</th>
<th>Reference</th>
<th>SCIT/controls, n</th>
<th>Allergen (manufacturer)</th>
<th>Dose</th>
<th>Duration</th>
<th>Positive outcomes (SCIT vs controls)*</th>
<th>Negative outcomes</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBPC</td>
<td>Peroni et al. [9]</td>
<td>12/11 (asthma)</td>
<td>HDM (Alpare, by DHS)</td>
<td>800 alpare units</td>
<td>12 mo</td>
<td>SCIT: skin reactivity</td>
<td>SCIT and placebo: improved PC&lt;sub&gt;20&lt;/sub&gt;, MCh, PC&lt;sub&gt;20&lt;/sub&gt; HDM</td>
<td>All children moved to Italian Alps for 1 y; both groups improved</td>
</tr>
<tr>
<td>DBPC</td>
<td>Adkinson et al. [10]</td>
<td>121 (moderate to severe asthma)</td>
<td>Allergen mix—maximum of 7</td>
<td>?</td>
<td>18–24 mo</td>
<td>No statistically significant reduction in medication use, days taking oral CS, PC&lt;sub&gt;20&lt;/sub&gt;</td>
<td>Both groups improved; no intergroup differences</td>
<td></td>
</tr>
<tr>
<td>DBPC</td>
<td>Hedlin et al. [11]</td>
<td>15/14 (asthma)</td>
<td>Cat or HDM (Alutard, by ALK-Abelló)</td>
<td>15 μg Fed d 1 or 7 μg Der p 1</td>
<td>36 mo</td>
<td>PC&lt;sub&gt;20&lt;/sub&gt; allergen; symptoms of cat exposure; IgG4 rise</td>
<td>SCIT: PC&lt;sub&gt;20&lt;/sub&gt; histamine improved (non-statistically significant); mediation</td>
<td>All children also received pollen SCIT</td>
</tr>
<tr>
<td>DBPC</td>
<td>Wang et al. [12]†</td>
<td>44/41 (asthma)</td>
<td>HDM (Alutard, by ALK-Abelló)</td>
<td>7 μg Der p 1</td>
<td>12 mo (phase 1: 6 mo, build-up; phase 2: 6 mo, maintenance)</td>
<td>Phase 2: symptoms, skin reactivity</td>
<td>No statistically significant reduction in medication use, morning and evening PEF, PC&lt;sub&gt;20&lt;/sub&gt; histamine, IgE HDM, ECP, blood eosinophils</td>
<td>All patients on ICS; intragroup: significant reduction in symptoms + medication; pediatric subgroup: non-statistically significant differences between SCIT and placebo groups</td>
</tr>
<tr>
<td>RCT</td>
<td>Pifferi et al. [13]</td>
<td>15/14 (asthma)</td>
<td>HDM; (Conjuvac; DHS and Bayer SpA [Milan, Italy])</td>
<td>? (in-house reference extract)</td>
<td>36 mo</td>
<td>Asthma exacerbations, use of bronchodilators and oral corticosteroids; PC&lt;sub&gt;20&lt;/sub&gt; MCh; new sensitizations</td>
<td>Medication score, lung function (non-statistically significant improvement), SPT</td>
<td></td>
</tr>
<tr>
<td>RCT</td>
<td>Tsai et al. [14]</td>
<td>30/30 (asthma)</td>
<td>HDM</td>
<td>?</td>
<td>12 mo</td>
<td>Asthma symptoms</td>
<td></td>
<td>Mechanistic study (see text)</td>
</tr>
<tr>
<td>RCT</td>
<td>Jacobsen et al. [20••]</td>
<td>103/102 (allergic rhinitis, mild asthma)</td>
<td>Birch or grass (Alutard, by ALK-Abelló)</td>
<td>12 μg Bet v 1 or 20 μg Phl p 5</td>
<td>36 mo</td>
<td>7 y post-SCIT: asthma development, rhinoconjunctivitis symptoms, conjunctival provocation test; 2 y post-SCIT: same + PC&lt;sub&gt;20&lt;/sub&gt; MCh, SPT</td>
<td>7 y post-SCIT: PC&lt;sub&gt;20&lt;/sub&gt; MCh, SPT</td>
<td>79/68 patients were studied at 7-y follow-up</td>
</tr>
</tbody>
</table>

*Positive results are only those showing a statistically significant difference between groups.
†Eighty-five of the 132 patients were children.
‡Forty-five patients were treated with depot preparations from ALK-Abelló, Stallergènes, or Allergopharma, and 40 patients were treated with aqueous extracts from Greer Laboratories.
CS—corticosteroids; DBPC—double-blind, placebo-controlled trial; DHS—Dhome-Hollister-Stier (Bridgend, United Kingdom); ECP—eosinophil cationic protein; HDM—house dust mite; ICS—inhaled corticosteroids; IL—interleukin; MCh—methacholine; MCP-1—monocyte chemoattractant protein-1; NO—nitric oxide; OCT—open controlled trial; PEF—peak expiratory flow; RCT—randomized, controlled trial; SCIT—subcutaneous immunotherapy; SPT—skin prick test; TGF-ß—transforming growth factor-ß; TU—treatment units.
<table>
<thead>
<tr>
<th>Design</th>
<th>Reference</th>
<th>SCIT/controls, n</th>
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<th>Dose</th>
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<th>Negative outcomes</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCT</td>
<td>Eng et al. [17]</td>
<td>14/14 at start; 13/10 in 1997</td>
<td>Pollen (Allergovit, by Allergopharma)</td>
<td>Allergoid</td>
<td>Every 3 mo/y for 3 y</td>
<td>1997: eye, nose, chest, and total symptoms; SPT; percentage of patients with seasonal asthma symptoms; new sensitizations</td>
<td>No statistically significant reduction in medication use ($P = 0.08$), conjunctival provocation (non-statistically significant tendency)</td>
<td>SCIT given 1989–1991; patients prospectively observed during 1997 and 2003 pollen seasons</td>
</tr>
<tr>
<td>OCT</td>
<td>Eng et al. [18]</td>
<td>14/14 at start; 12/10 in 2003</td>
<td>Pollen (Allergovit, by Allergopharma)</td>
<td>Allergoid</td>
<td></td>
<td>2003: symptoms, medication, symptom and medication score; new sensitizations</td>
<td>SPT, seasonal asthma ($P = 0.08$)</td>
<td></td>
</tr>
<tr>
<td>OCT</td>
<td>Keskin et al. [19]</td>
<td>27/26 (severe allergic rhinitis)</td>
<td>Pollen (Allergovit, by Allergopharma)</td>
<td>20-μg Phl p 5 equivalent (6000 TU)</td>
<td>30 mo</td>
<td>12 mo: rhinitis + asthma symptoms reduced, medication; seasonal reduction: $PC_{20}$, $MCh$ blunted; seasonal IgE rise; nasal provocation; IgE/IgG ratio reduced; IL-4 reduced</td>
<td>Reduced ECP rise in season (non-statistically significant); IL-10, TGF-β augmented in both groups</td>
<td>Allergoid; many seasonal immunologic alterations blunted with SCIT</td>
</tr>
<tr>
<td>OCT</td>
<td>Cevit et al. [24]</td>
<td>19/12 (asthma)</td>
<td>HDM (Allergovit, by Allergopharma)</td>
<td>5000 TU</td>
<td>12 mo</td>
<td>Symptoms</td>
<td>No statistically significant reduction in medication use, serum NO, ECP, and $MCP-1$ levels, $PC_{20}$ HDM, SPT</td>
<td>SCIT group (baseline–12 mo): significant reduction in medication, NO, ECP, MCP-1, $PC_{20}$ HDM</td>
</tr>
<tr>
<td>OCT</td>
<td>Inal et al. [23]</td>
<td>85/62* (allergic rhinitis, asthma)</td>
<td>HDM (Greer Laboratories, ALK-Abelló, Stallergènes, Allergopharma)</td>
<td>Approximately 0.5–5.0 μg Der p 1</td>
<td>5 y</td>
<td>SCIT: 75% no new sensitizations vs 47% for controls ($P = 0.002$)</td>
<td></td>
<td>Patients with new sensitizations had higher atopy and medication scores</td>
</tr>
<tr>
<td>Retro-</td>
<td>Cools et al. [16]</td>
<td>48/42 (asthma)</td>
<td>HDM, grass (HAL Allergy)</td>
<td>5–20 μg Der p 1 and Der p 2</td>
<td>61 mo</td>
<td>Asthma symptoms</td>
<td>Less medication (non-statistically significant), SPT, $PC_{20}$ MCh</td>
<td>Evaluation 9.3 years after SCIT in childhood</td>
</tr>
</tbody>
</table>

*Positive results are only those showing a statistically significant difference between groups.

†Eighty-five of the 132 patients were children.

‡Forty-five patients were treated with depot preparations from ALK-Abelló, Stallergènes, or Allergopharma, and 40 patients were treated with aqueous extracts from Greer Laboratories.

CS—corticosteroids; DBPC—double-blind, placebo-controlled trial; DHB—Dhome-Hollister-Stier (Bridgend, United Kingdom); ECP—eosinophil cationic protein; HDM—house dust mite; ICS—inhaled corticosteroids; IL—interleukin; MCh—methacholine; MCP-1—monocyte chemoattractant protein-1; NO—nitric oxide; OCT—open controlled trial; PEF—peak expiratory flow; RCT—randomized, controlled trial; SCIT—subcutaneous immunotherapy; SPT—skin prick test; TGF-β—transforming growth factor-β; $TU$—treatment units.
improved in the SCIT group. No microgram major allergen dose was stated.

Another RCT [14] with house dust mite SCIT for 12 months in asthmatic children showed reduced asthma symptoms ($P < 0.05$, active vs control), but again no dose was mentioned. Mechanistic assays were also carried out in this study.

Open controlled trials of SCIT in children

Long-term efficacy

The first publication on the long-term efficacy of hypo-sensitization therapy for bronchial asthma in children was the classic study by Johnstone and Dutton [15] in 1968, when as-yet-unstandardized extracts were used.

Cools et al. [16] examined the effect of monthly injections of house dust mite or pollen over 5 years in asthmatic children. The house dust mite extract contained 5000 allergy units (AU) (Allerset, HAL Allergy, Haarlem, The Netherlands [5000 AU = 5–20 μg of Der p 1 and Der p 2]). At re-evaluation almost 10 years later, the risk of frequent asthmatic symptoms was three times higher in the control group than in the SCIT-treated group (prevalence ratio, 3.43; $P = 0.0006$). The frequent use of antiasthmatic medication was also more pronounced in the control group, although the difference was not statistically significant ($P = 0.38$). Lung function parameters and results of skin prick tests with house dust mite were comparable in the two groups.

Eng et al. [17,18] recently demonstrated the long-term benefit of SCIT, 6 and 12 years after termination. From 1989 to 1991, preseasonal SCIT was given with a depot allergoid pollen preparation (Allergovit; Allergopharma, Rheinbeck, Germany) for 3 consecutive years. Fourteen children with severe seasonal allergic rhinitis (mean age, 9.3 years) were recruited, and 14 matched controls, who refused SCIT, were sought. Six and 12 years after termination of SCIT, 12 actively treated patients and 10 controls were found for the follow-up study. Between-group comparisons showed a continued reduction in seasonal rhinitis symptoms ($P < 0.03$), medication use ($P = 0.05$), and new sensitizations ($P = 0.05$). Moreover, at 6 years, a reduction in skin prick sensitivity was seen, but this reduction was lost after 12 years. The same pattern was shown for seasonal asthma: still reduced at 6 years but with only a trend toward reduction at 12 years post-SCIT ($P = 0.087$, asthma was still present in 33% of the active group vs 70% of the controls). No precise dose of the allergoid extract was stated in this publication, but the same product 12 years later was reported to have 20-μg group 5 major allergen equivalents in the last measurable production step before the extracts were formed into an allergoid [19].

The only RCT on the long-term efficacy of SCIT and prevention of asthma is the preventive allergy treatment (PAT) study [20••]. Between 1992 and 1994, 205 children 6 to 14 years old with seasonal allergic rhinitis to birch and/or grass pollen were stratified and randomized to the SCIT or control group. The maintenance SCIT treatment consisted of injections of a depot preparation (100,000 SQ-U/mL) with 20 μg Phl p 5 and/or 12 μg of Bet v 1 every 6 weeks for 3 years. At the beginning of the trial, 117 children had no seasonal asthma symptoms. Significantly fewer actively treated patients had developed asthma 7 years post-SCIT as evaluated by clinical symptoms (odds ratio, 2.5 [1.1–5.9]). Rhinitis and conjunctivitis symptoms and conjunctival provocation test still favored the actively treated individuals, showing a statistically significant difference compared with the control group. However, skin reactivity had returned.

New sensitizations

The first publication to report on the reduction in new sensitizations in children with SCIT treatment dates from 1997 [21]. Forty-four asthmatic children were included in this open controlled study and received house dust mite SCIT (Stallergènes, Antony, France, dose not stated) for 3 years. New sensitizations developed in 12 of 22 of the actively treated children and in the entire control group.

Pajno et al. [22] reported similar findings in asthmatic children at 6-year follow-up after SCIT with a house dust mite extract started circa 1993 to 1994 (ALK-Abelló, Milan, Italy, dose not stated).

A more recent open controlled study on the same subject [23] was carried out in 147 children (45 depot preparation SCIT, 40 aqueous solution SCIT, 62 controls). At the end of 5 years of SCIT, 75.3% of the patients in the SCIT groups showed no new sensitization, compared with 46.7% in the control group ($P = 0.002$). No differences were observed between the aqueous and depot SCIT subgroups. The patients developing new sensitizations had higher atopy ($P = 0.002$) and medication scores for rhinitis ($P = 0.008$) and asthma ($P = 0.013$) compared with patients not developing new sensitizations after 5 years of SCIT. Extracts from various manufacturers were used in the depot preparation group (ALK-Abelló, Stallergènes, Allergopharma), which resulted in doses varying from approximately 0.5 to 5 μg Der p 1. In the aqueous SCIT group, doses were adjusted individually from a 5000-AU/mL D. pteronyssinus extract (Greer Laboratories, Lenoir, NC).

The open controlled study by Keskin et al. [19] was very comprehensive, measuring clinical and immunologic outcomes. The extract used was an allergoid with major allergen equivalent in the last measurable production step (before allergoidization) of 20 μg Phl p group 5 per maintenance dose (aluminium-adsorbed Allergovit). Rhinitis and asthma symptoms and medication scores were significantly better in the active group (27 children) than in the control group (26 children) after 2.5 years of SCIT. Moreover, immunologic parameters improved.

Immunologic mechanisms and provocation tests with SCIT treatment in children

Tsai et al. [14] studied apoptosis of certain cell lines in an RCT of SCIT with a house dust mite extract (dose and
manufacturer not stated) in 60 asthmatic children. Using the TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling) method, the investigators showed, after 12 months of SCIT, an augmented apoptosis of CD4+ intereleukin (IL)-4+ T-helper type 2 cells ($P = 0.001$) and of CD45+ R0 cells ($P < 0.05$) in comparison with the control group, whereas the apoptosis of CD4+ interferon (IFN)-γ T-helper type 1 cells was unchanged. Moreover, specific serum IgE was reduced, and IFN-γ was augmented with statistical significance.

Another 12-month, open controlled study [24] of house dust mite SCIT (5000 treatment units/mL of Dpt/ Df, no microgram dose stated) in 31 asthmatic children showed a reduction of eosinophil cationic protein (ECP), nitric oxide, monocyte chemoattractant protein-1, and skin prick test reactivity in the treated group. However, specific bronchial hyperreactivity (PC$_{20}$ allergen) did not show any difference, as it improved in both groups.

In the open controlled study by Keskin et al. [19] (20 μg Phl p 5 allergoid, see above), a statistically significant improvement was demonstrated in the seasonal reduction of PC$_{20}$ methacholine, skin test reactivity, seasonal nasal reactivity (nasal provocation testing for grass pollen), and nasal lavage ECP levels in the active group compared with the controls. Changes were recorded after the first year of immunotherapy but were more pronounced after the second year. The seasonal increase in IgE decreased ($P < 0.05$), and grass-specific IgG, IgG1, and IgG4 already had increased significantly at the end of the seven-injection build-up therapy ($P < 0.001$ for all). IL-4 levels in the culture supernatants showed a steady decline from baseline at first and second year of immunotherapy ($P < 0.001$).

Another open controlled SCIT study also showed a rise in specific serum IgG and a reduction in specific serum IgE in 56 children with seasonal asthma, but again no dose was stated [25].

Clinical efficacy studies for SLIT in children
All randomized efficacy studies up to now have been conducted in children over the age of 5 years.

Double-blind, placebo-controlled and randomized controlled trials with SLIT in children
SLIT studies have been published since 1986 [26], with the first on SLIT in children in 1990 [27]. The first pediatric study to mention dose used in micrograms of major allergen was the DBPC study published in 1997 by Hirsch et al. [28].

Various meta-analyses on SLIT have been conducted since, two of them in children. However, the SLIT studies carried out in children vary greatly in many parameters. This makes the heterogeneity in the meta-analyses too high [29]—with an I² of 85% to 95%—which strongly reduces the validity of the results obtained.

As a solution for the heterogeneity in meta-analysis, systematic reviews can be carried out. Some on SLIT in children have been published recently [30,31], one of the most complete being the systematic review by Röder et al. [30], as both DBPC trials and RCTs on SLIT for allergic rhinitis were included and a solid quality assessment was made.

Röder et al. [30] concluded that there was no evidence for effect of SLIT in children, which may be a valid conclusion based on their data. However, the authors did not include articles published after June 2006. Moreover, a closer look at the individual studies in their systematic review shows us interesting data that may explain the negative outcomes in some SLIT studies.

From June 2006, when the Röder et al. [30] review ended, until June 2008, various trials on SLIT in children have been published. Three large, high-quality (> 6 points on the Delphi list [32]) DBPC RCTs have been conducted in children, two with grass pollen SLIT tablets [33••,34••] and one DBPC, multiple-dose study with birch SLIT [7••]. In the first DBPC trial [33••], 75,000 standard quality tablet (SQ-T) (Grazax; ALK-Abelló, Horsholm, Denmark, 15 μg Phl p 5) were given daily in a pre-coseasonal protocol, started 17 weeks preseasonally. Results were given for 114 SCIT-treated and 120 placebo group patients (5–16 years old). Statistically significant reductions in the symptoms and medication scores were demonstrated (22% and 34%, respectively; 28% and 65% in peak pollen season), together with a 64% reduction in asthma symptoms.

In the second DBPC trial [34••], 278 children (5–17 years old) with seasonal allergic rhinoconjunctivitis were recruited. A total of 227 patients could be included in the per-protocol analyses (115 SLIT, 112 placebo). Daily 300 index of reactivity (IR) tablets of five grasses were administered (Oralair; Stallergènes, Antony, France, 25-μg group 5 major allergen), with a 2-day run-in period of 100 IR (day 1) and 200 IR (day 2). After a pre-coseasonal treatment started 4 months before the pollen season, a statistically significant improvement was documented in the primary efficacy variables: symptoms and medication score.

The only dose–effect study of immunotherapy in children [7••] was done with two different doses of birch pollen extract (Aquagen SQ; ALK-Abelló, Horsholm, Denmark). The 32 children in the low-dose group (1.6 μg Bet v 1, 5 d/wk) showed statistically significant reduction only in symptoms, whereas the 27 children in the high-dose group (13 μg Bet v 1, 5 d/wk) improved in symptoms and medication scores compared with placebo.

Trials with SLIT for allergic asthma in children
Apart from the presented trials on SLIT for allergic rhinitis, there are some DBPC trials on SLIT with house dust mite extract in children with asthma [22,35,36,37••,38]. In these studies, the relative dose in monthly micrograms of Der p 1/2 varied from 1.5 to 120 times the recommended SCIT dose. No clear relationship was found.
between dose and effectiveness of SLIT in these studies, as some low-dose trials showed improvement and some high-dose studies were negative.

Finally, there was one more negative study of SLIT for allergic respiratory symptoms in a primary care setting. This study is not mentioned any further, as children were not selected on the basis of a positive skin prick test or treated by specialists.

More details of individual studies on SLIT in pediatric allergic rhinitis and asthma can be found in a previous issue of this journal [39•].

Immunologic mechanisms and provocation tests with SLIT treatment in children

Just as in the adult studies on immunologic changes with SLIT, increases in serum specific IgE, IgG4, IL-10, and transforming growth factor-β have been demonstrated in children, as has been a reduction in eosinophils and mRNA for IL-5 in stimulated peripheral blood mononuclear cells [40]. Recently, an upregulation of various IFN-γ-stimulating cytokines, such as IL-18 and signaling lymphocytic activation molecule, was detected. Some of these changes have been shown to be dose- and efficacy-related [40,41]. In low-dose SLIT studies, changes are not seen [42,43] or are not statistically significant [44].

Safety of immunotherapy in children

**SCIT safety**

Akcakaya et al. [45] reviewed all records of 88 patients 6 to 15 years old who had been treated with SCIT from 1989 to 1997. Of a total of 5760 injections given, 206 injections (3.57%) caused local reactions, and 12 patients presented with systemic reactions (0.2%), one of which was classified as anaphylaxis with a drop in blood pressure and respiratory distress leading to intubation. Four patients received epinephrine. Seven of the 12 patients (58.3%) experienced no local reactions before a systemic reaction, and in this study, all systemic reactions occurred less than 30 minutes after the injection. Doses were usually increased weekly. The typical maximum dose was 0.8 mL of 0.01 weight/volume extract of Stallergènes and 1 mL of 0.01 weight/volume extract of Novo-Helisen (Allergopharma, Rheinbeck, Germany), Alutard, and Bencard (Bencard Laboratories, Brentford, England). Once the patient reached the maintenance dose or the maximum tolerated dose, the injection schedule was changed to every 2 and then to every 4 weeks.

Keskin et al. [46] investigated the safety of SCIT in asthmatic children, looking at nonspecific bronchial hyperreactivity 1 hour after an immunotherapy injection. The authors found no reduction in the PC_{20} with methacholine after SCIT administration (dose not stated).

**SLIT safety**

In general, SLIT is considered to have less systemic adverse events than SCIT. Even so, systemic adverse events have been reported to occur in the trials on SLIT, sometimes leading to treatment discontinuation [6]. In adults, a dose–response relationship in adverse events has been shown with SLIT grass tablets with doses up to 1.000.000 SQ-T, which contain approximately 200 μg Phl p 5 (13 times the dose given normally) [47]. In a 1-month safety study of the grass tablet with 15 μg Phl p 5, of 60 patients 5 to 16 years old, one child presented with a serious adverse event—an asthma attack—and another presented with various systemic, nonserious adverse events. Both were withdrawn from the study [48•].

With ever-increasing use of this treatment modality, the first serious adverse events in the open population have been published, two in the pediatric age group [49,50••]. The second pediatric case was a 16-year-old female, who after a 3-week break in maintenance treatment in the third year of SLIT decided to take six times her normal dose (normal dose was 10 drops of 100 IR/mL, total dose ~ 51 μg Der p 1). An anaphylactic reaction started at 5 minutes and evolved into shock, but with a good final outcome.

Conclusions

In all pediatric immunotherapy studies reviewed, the same dose is used as in adults (in SCIT and SLIT). Studies with varying design have been presented in this review on SCIT in children. In some, the dose administered is stated in micrograms of major allergen. Almost all SCIT studies have been carried out with depot preparations (different from the preparations used in the United States). Aluminium-adsorbed preparations permit applications at maintenance every 6 to 8 weeks. Moreover, this is a slightly T-helper type 1–deviating adjuvant. All SCIT studies use similar doses per application: for grass SCIT, 20 μg group 5 major allergen; for birch SCIT, 12 μg Bet v 1; for cat, 15 μg Fel d 1. For house dust mite, the doses reported vary from 0.5 μg Der p 1 in one study to 5 to 20 μg Der p 1 in all other SCIT trials. Mixes of up to seven allergens do not seem to be effective. The long-term studies conducted many years after SCIT discontinuation are very promising. An interesting issue is that although the skin prick tests’ positivity seems to return 5 to 6 years after discontinuation of SCIT in these two trials, clinical efficacy partially remains.

The situation is different for SLIT. Since the first SLIT publication more than 20 years ago, the field has been marked by dose searching, in quantity and in frequency and formulation. Some good trials published from 2006 to the present make certain aspects of dosing clearer. This is pointed out as we go through the negative studies in the Röder et al. [30] review below:

1. Dose. The most recent SLIT studies in adults [51••,52••] and children [7••] demonstrate a clear dose–response relationship.
2. Dosing frequency. Daily dosing tends to be more effective than dosing with lower frequency [51••,52••,53].

3. Preseasonal dosing. Commencing SLIT at least 4 months before the start of the pollen season augments its efficacy [54]. This was not the case in three of the eight seasonal studies.

4. Patient selection, asthma. Two of the studies focused on asthma. In these studies, rhinitis symptoms were secondary (or exploratory) end points, likely with insufficient power to detect a statistically significant difference between groups.

5. Duration of treatment. Four of 12 studies had interrupted treatment. In the latest Cochrane review on SLIT, the best effect was seen in studies with at least 1 year of continuous treatment [55•].

6. Patient selection, polysensitization. In the study conducted by Vourdas et al. [56], polysensitized children were included. In the SLIT group, 30 of 34 children were polysensitized, 23 of them to grass. The olive pollen season starts in May, showing important overlap with the grass pollen season that starts just 1 month earlier in Greece. Of the placebo patients, only 15 of 32 were sensitized to grass. Taking a closer look at the figures on symptom scores, it becomes clear that during the first year, the SLIT group showed more symptoms than the placebo group, whereas during the second year, this relationship was reversed. Although statistics did not show a difference between groups, with these reservations, a beneficial treatment effect seems likely.

7. Number of patients with allergic rhinitis included. More than one half of the studies were underpowered, with less than 20 allergic rhinitis patients in the actively treated group.

8. Possible confounding by drug effects. In one study, all patients received maintenance inhaled fluticasone during the pollen season in addition to SLIT/placebo, which may have resulted in marked improvement in SLIT and placebo groups, thereby reducing intergroup differences for symptoms (P = 0.059 for nasal symptoms).

When these points are all overcome, SLIT does show clinical efficacy, as has been demonstrated in the recent DBPC pollen SLIT studies [7••,33••,34••]. These studies are consistent and present a high level of evidence for SLIT’s effect in pollen-induced rhinoconjunctivitis in children.

The evidence for SLIT’s effect in perennial asthmatic children with house dust mite allergy is still not so straightforward, as two of four recent, high-quality studies were negative [37••,57]. One problem in this pathology is likely the multifactorial cause of asthma. Especially in perennial asthma, sensitization to house dust mite may be found without it being the causal factor. Asthma has many faces, one of them being an alteration in the repair mechanisms of the bronchial epithelium that will not improve with immunotherapy. Here, the patient selection will have to be more specific, eliciting only those patients in whom asthma is mainly caused by their allergy. Moreover, the use of inhaled corticosteroids in treatment and control groups in all SLIT asthma studies may mask a rise in symptoms in the placebo group. Randomized, long-term studies in SLIT are still awaited.

With doses that are too high, systemic and even serious adverse reactions do occur in SLIT, making safety a determining factor for the maximum dose. This maximum tolerated dose does not seem to be lower for small children in comparison with older ones [58,59•].

In conclusion, positive and negative clinical trials with immunotherapy in children have taught us to come closer to an optimal dose, improving efficacy, but the last word has not yet been spoken, especially in regard to SLIT for asthma and SLIT’s long-term efficacy. Moreover, SLIT has to be a specialist’s treatment given that the patient’s selection is crucial to obtain efficacy, and adverse events, although rare, do occur.

Disclosure
Dr. Larenas-Linnemann has served on the speakers’ bureau for Schering-Plough and Merck Sharp & Dohme and has received support for assisting US Congress from Schering-Plough, Merck Sharp & Dohme, and ALK-Abelló.

References and Recommended Reading
Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

Review of mechanisms of immunotherapy.


Review of different methods of potency expression of allergen extracts per allergen manufacturer in Europe.

Project to improve unification of in vitro tests and reagents to measure allergen extract potency.


The only dose–response SLIT study in children.


Twelve years after subcutaneous discontinuation, efficacy can still be shown.


Seven years after discontinuation of SCIT, still fewer children developed asthma.


Large DBPC trial on SLIT in pollen-allergic children showing efficacy for symptoms and medication.


Another large DBPC trial on SLIT in pollen-allergic children showing efficacy for symptoms and medication.


Negative house dust mite SLIT study in asthmatic children, although the set-up seems good.


Documented anaphylaxis with SLIT in a 16-year-old female taking six times her maintenance dose after a 3-week suspension.