

## Current reviews of allergy and clinical immunology

Series editors: Donald Y. M. Leung, MD, PhD, and Dennis K. Ledford, MD

### Allergen immunotherapy: Where is it now?

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The scientific basis and the proof of clinical effectiveness of allergen immunotherapy administered by subcutaneous injection (SCIT) are well established. It is effective treatment for sensitivity to *Hymenoptera* venom and for allergic rhinitis and allergic asthma. SCIT administered in the proper setting reduces the development of new sensitivities and progression from rhinitis to asthma. Further, the beneficial effects persist long after completion of a course of treatment. Although many people enjoy the benefits of SCIT, extension of its use to the many others who might be candidates for this treatment is limited by its drawbacks of safety concerns and the inconvenience of repeated clinic visits over several years to receive the injections. There are many attempts underway to improve on the safety and convenience while still retaining the benefits of SCIT. These include approaches using current allergen extracts, especially by administering them sublingually. Alternatively, through recombinant technology, extracts are being modified to reduce their allergenicity without reducing their immunogenicity. They are being linked to immunostimulatory DNA sequences that will modify their *in vivo* processing resulting in an enhanced nonallergic response or they are being incorporated into fusion proteins with inhibitory properties for mast cells and basophils. (*J Allergy Clin Immunol* 2007;119:769-77.)

**Key words:** Immunotherapy, SCIT, SLIT, sublingual, subcutaneous, asthma, rhinitis

As the practice of allergen immunotherapy approaches its 100th anniversary, major changes appear likely in the way it is practiced. The scientific rationale for immunotherapy has never been stronger. However, extension of the use of

#### Abbreviations used

SCIT: Subcutaneous immunotherapy

SLIT: Sublingual immunotherapy

allergen immunotherapy to the many appropriate patients is hampered by 2 continuing drawbacks: concerns for safety and, because of these real concerns, the inconvenience of the treatment schedules used. In response to these drawbacks, attention is being given to developing safer and more convenient ways to administer the currently available allergen extracts or to making modifications in the allergen extracts, rendering them inherently safer and more convenient. It will be important, however, not to sacrifice the effectiveness of the current method of administering immunotherapy while seeking greater safety and convenience. As a background, this article reviews where we are in 2007 with immunotherapy using subcutaneous injections of unmodified extracts in aqueous or glycerin solutions with or without adsorption to alum. We then consider the alternative approaches that have advanced to clinical application, particularly the administration of allergen extracts sublingually.

#### SUBCUTANEOUS IMMUNOTHERAPY

##### Established aspects

*Diseases or conditions in which subcutaneous immunotherapy is effective.* Currently subcutaneous immunotherapy (SCIT) is established as effective treatment for patients with IgE-mediated reactions to hymenoptera venom,<sup>1</sup> allergic rhinitis,<sup>2</sup> and allergic bronchial asthma.<sup>3</sup>

*Effective doses of allergen extract.* A problem in discussing doses for immunotherapy is the many ways allergen extract strength is expressed, some of which, such as weight by volume and protein nitrogen units, have no relation to allergenic potency—that is, the quantity of allergenic proteins per unit volume of extract. Even when relevant measures are used, such as the reaction on skin testing of subjects with allergy or *in vitro* inhibition assays, the units employed to express the results are often unique to a particular company or regulatory agency and

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**TABLE I.** Immunotherapy dosing by major allergen content

Allergen extract	Major allergen	Effective dosing	Less effective or ineffective dosing
Short ragweed	Amb a 1	6 <sup>5</sup> to 24 <sup>6</sup> µg	0.6 <sup>7</sup> and 2 <sup>6</sup> µg
Timothy	Phl p 5	15 <sup>8</sup> & 20 <sup>9</sup> µg	2 <sup>8</sup> µg
Birch	Bet v 1	3.28 <sup>10</sup> & 12 <sup>11</sup> µg	ND
<i>Dermatophagoides pteronyssinus</i>	Der p 1	7 <sup>12</sup> & 12 <sup>13</sup> µg	0.7 <sup>14</sup> µg
<i>Dermatophagoides farinae</i>	Der f 1	10 <sup>15</sup> µg	ND
Cat dander	Fel d 1	11 <sup>16</sup> , 17 <sup>17</sup> µg	0.6 <sup>18,19</sup> and 3 µg <sup>18,19</sup>
Dog dander	Can f 1	15 <sup>20</sup> µg	0.6 <sup>20</sup> & 3 <sup>20</sup> µg
<i>Alternaria</i>	Alt a 1	1.6 <sup>21</sup> µg	ND

ND, Not determined.

**TABLE II.** Major allergen content: US standardized and unstandardized extracts

Extract	Expressed concentration	Major allergen	Mean major allergen content
Short ragweed*	1:10 wt/vol	Amb a 1	424 µg/mL
Timothy grass*	100,000 BAU/mL	Phl p 5	680 µg/mL
Kentucky blue grass*	100,000 BAU/mL	Group 5	300 µg/mL
Orchard grass*	100,000 BAU/mL	Group 5	750 µg/mL
Bermuda grass*	10,000 BAU/mL	Group 1	300 µg/mL
<i>Dermatophagoides pteronyssinus</i> *	10,000 AU/mL	Der p 1	76 µg/mL
<i>Dermatophagoides farinae</i> *	10,000 AU/mL	Der f 1	56 µg/mL
Cat dander*	10,000 BAU/mL	Fel d 1	43 µg/mL
AP dog dander†	1:100 wt/vol	Can f 1	180 µg/mL

AP, Acetone precipitated (product of Hollister-Stier Laboratories, Spokane, Wash); AU, allergy units; BAU, bioequivalent allergy units.

\*Personal communication, ALK Laboratories, Round Rock, Tex, July 2006.

†Personal communication, Hollister-Stier Laboratories, Spokane, Wash, November 2006.

are not interconvertible. Furthermore, measures of total allergenic potency are unable to detect differences in the relative amounts of the major allergens within the extract. For all the shortcomings in the use of major allergen content to express extract potency and dosing,<sup>4</sup> it remains the only international language currently available. Double-blind, placebo-controlled studies have been conducted with extracts of all of the allergens currently standardized in the United States, although only a few have been conducted with the US extracts. The effective dose may be expressed as the quantity of the major allergen delivered as a maintenance dose (Table I).<sup>5-11,13-21</sup> Expressed in this way, the range of doses is seen to be relatively narrow (3-20 µg major allergen), with the exception of *Alternaria*, for which significant improvement was achieved with a dose containing only 1.6 µg of the major allergen Alt a 1.<sup>12</sup> Information on dose responses is available for most of the extracts (Table I). In general, a dose 1/5 to 1/10 of the proven effective dose has been less effective or ineffective in eliciting the responses being assessed (Table I). Because, of the US standardized extracts, only cat and ragweed are standardized by major allergen, the content of major allergen varies quite widely for extracts of the same expressed potency. Nevertheless, information on the mean major allergen content of an extract is often available from the extract manufacturer (Table II). The markedly lower major allergen content of cat and house dust mite extracts is reflected in their lower Allergen or

Bioequivalent Allergen Unit rating by the US system of expressing standardized extract potency. Because approximately the same amount of major allergen is required for effective immunotherapy with these weaker extracts (Table I), it follows that larger volumes of these extracts must be either administered as monotherapy or compounded into a treatment set (Table III). Studies of dosing with nonstandardized extracts are not performed because the results could not be applied to other manufacturers' extracts or even to other lots from the same manufacturer. However, on the basis of a limited number of analyses, it appears that most nonstandardized pollen extracts are of similar potency to the standardized ragweed and grasses and may be dosed accordingly.<sup>22</sup> On the other hand, most dog dander (personal communication, ALK Laboratories, July 2006), cockroach,<sup>23</sup> *Alternaria*, and *Aspergillus*<sup>24</sup> extracts are quite low in major allergen content and, when tested, in overall allergenic potency.

**Prevention of disease progression.** Although the clinical response to immunotherapy has been proven to be allergen-specific,<sup>25,26</sup> there is now good evidence that administration of appropriate monotherapy to monosensitized patients can reduce the likelihood of the patients developing additional sensitivities.<sup>27-29</sup> In the 2 largest studies,<sup>28,29</sup> the likelihood of developing additional positive skin tests was reduced from about 2/3 to 1/4, and further, the protection was shown to persist 3 years after the 3-year to 4-year course of specific immunotherapy was completed.

**TABLE III.** Effective dosing with US extracts

Extract	Major allergen dose required	Add to 10 mL vial, assuming 0.5-mL maintenance injection
Timothy grass	20 µg Phl p 5	0.6 mL 100,000 BAU/mL
Short ragweed	12 µg Amb a 1	0.8 mL 1:10 wt/vol
<i>Dermatophagoides pteronyssinus</i>	7 µg Der p 1	2.2 mL 10,000 AU/mL
<i>Dermatophagoides farinae</i>	10 µg Der f 1	3.3 mL 10,000 AU/mL
Cat dander	15 µg Fel d 1	6 mL 10,000 BAU/mL
Dog dander	15 µg Can f 1	2.1 mL 1:100 wt/vol AP dog (Hollister-Stier Laboratories)

AP, Acetone precipitated; AU, allergy units; BAU, bioequivalent allergy units.

Three years of immunotherapy with birch and/or timothy in children with only seasonal allergic rhinitis was found to reduce the likelihood of their developing asthma by a factor of approximately 2.5.<sup>30</sup> Follow-up reports 2<sup>31</sup> and 7<sup>32</sup> years after stopping immunotherapy have shown persistence of the reduced risk for developing asthma.

*Persistence of effect after discontinuation.* Given the persistence of the protective effect after discontinuing immunotherapy on the development of new sensitizations and progression to asthma, it is not surprising that there is also persistence of reduction of clinical symptoms.<sup>33-35</sup> This has been shown most convincingly by a double-blind study in which subjects who had received 3 or 4 years of timothy grass immunotherapy were randomized to continue receiving monthly maintenance injections of grass extract or to receive placebo injections.<sup>35</sup> After 3 more years, there was no difference in control of symptoms or medication use between the 2 groups.

### Concerns and controversies

*Immunotherapy for atopic dermatitis and food allergy.* There have been a number of uncontrolled studies reporting improvement in atopic dermatitis with allergen immunotherapy.<sup>36</sup> Recently, a double-blind study of injection immunotherapy was conducted in 89 adults with chronic atopic dermatitis and sensitivity to house dust mites.<sup>36</sup> Maintenance doses of 20, 2000, and 20,000 standardized quality units were given weekly for 1 year. The top dose was 1/5 the customary maintenance dose for treating allergic respiratory diseases; the lowest dose could be considered a placebo. Fifty-one subjects completed the study, with those receiving the 2 higher doses showing significant reduction in symptom scores and topical steroid use.

Injection immunotherapy has also been tried for food allergy. Although patients with anaphylactic sensitivity to peanuts could tolerate larger doses of peanuts when they were receiving injections of peanut extract, the frequency of systemic reactions to the injections proved unacceptable.<sup>37</sup> However, in 3 studies, SCIT for birch allergy reduced symptoms of the oral allergy syndrome caused by eating apples.<sup>38-40</sup> The protective effect was lost over a period of 30 months after discontinuation of treatment.<sup>40</sup>

*Immunotherapy with multiple allergens in multiply sensitized patients.* All of the studies that have established

effective doses (Table I) have been conducted with a single allergen extract. It has been suggested that only single allergen immunotherapy is effective. However, the studies by Lowell and Franklin<sup>25</sup> and Franklin and Lowell<sup>41</sup> that established the effectiveness of immunotherapy involved manipulation of only the dose of ragweed in patients receiving extracts containing ragweed plus multiple other allergens. The classic study by Johnstone and Crump<sup>42</sup> demonstrated that immunotherapy containing higher doses of multiple allergens was effective in childhood asthma, whereas lower doses were less effective or without benefit.

*Safety.* Local, systemic, and even fatal reactions are a recognized complication of SCIT. Local reactions have not been found to be predictive of the subsequent occurrence of systemic reactions.<sup>43</sup> The exact incidence of reactions to immunotherapy is a function of patient sensitivity, the dose administered, and whether the extract is modified to delay absorption. Therefore, the multiple studies reporting the incidence of reactions have limited applicability. Two large studies that appear to be fairly representative of US practices made certain observations that may be generalizable.<sup>44,45</sup> Patients received treatment containing multiple allergens with individual allergen concentrations of 4000 protein nitrogen units/mL<sup>44</sup> or 1:100 wt/vol.<sup>45</sup> Systemic reactions were reported in 2.9%<sup>44</sup> and 2.1%<sup>45</sup> of patients. Both studies found no relation between pollen seasons and the incidence of systemic reactions. More systemic reactions did occur in highly sensitized patients.<sup>44</sup> The ultimate safety concern is the occurrence of a fatal reaction. These have been assessed by questionnaires sent to practicing allergists by the Immunotherapy Committee of the American Academy of Allergy, Asthma & Immunology.<sup>46,47</sup> The last 2 surveys, which encompassed the years 1985 to 2001, reported 34 fatalities, 33 from immunotherapy and 1 from skin testing to foods. Notably, 28 of 32 (88%) who experienced a fatal reaction had asthma that was labile, poorly controlled, or treated with oral corticosteroids in 21 of 28 (75%) of cases. Additional findings were that 18 of 32 (52%) occurred during the build-up phase, there were dosing errors in 5 (15%), 5 (15%) occurred with the first injection from a new vial, and 3 (9%) occurred with unsupervised injections.

*Dosing schedules.* Because of safety concerns, the rate of dose increase with conventional immunotherapy is gradual. With weekly injections, 8 to 12 months may

**TABLE IV.** Protease content of allergen extracts<sup>59</sup>

	Trypsin equivalent
Pollens	<1 $\mu$ g
Cat and dog dander	<1 $\mu$ g
House dust mites (US extracts)	<5 $\mu$ g
<i>Alternaria alternata</i>	29 $\mu$ g
American cockroach	168 $\mu$ g
<i>Aspergillus fumigatus</i>	212 $\mu$ g
<i>Penicillium notatum</i>	242 $\mu$ g

be required to reach the projected maintenance doses. Alternative, more rapid build-up to maintenance may be achieved by either rush or cluster schedules. With rush dosing, multiple injections are given daily, and maintenance is reached after 1 or several days. The price of this rapid achieving of maintenance is an increased rate of systemic reactions, which is reduced somewhat by premedication.<sup>48</sup> Cluster dosing consists of 2 or more injections per day, but visits are typically 1 or 2 days per week. Maintenance is not achieved as rapidly as with rush, but in comparison with conventional schedules, there does not appear to be an increased incidence of systemic reactions.<sup>49,50</sup>

**Immunotherapy with fungal extracts.** The importance of sensitivity to airborne fungal spores in causing severe attacks of asthma is well recognized.<sup>51-53</sup> Furthermore, a limited number of studies of immunotherapy conducted in Europe with extracts of *Cladosporium*<sup>54,55</sup> and *Alternaria*<sup>21</sup> have demonstrated therapeutic efficacy. However, these studies were not performed with the extracts commercially available in the United States, which have been shown to be of very variable allergen content and allergenic potency.<sup>56</sup> In addition to the questionable quality of available extracts, there are other problems in using fungal extracts for immunotherapy.<sup>57</sup> These include that there are hundreds of thousands of different species of fungi, and there is a lack of information on the degree of exposure for many of the fungi for which extracts are available. Furthermore, there is an absence of commercial extracts for many of the common fungi because they do not grow on artificial media.<sup>57</sup> Given these limitations, it has been recommended that immunotherapy to fungi be limited to patients with documented sensitive to a fungus, with symptoms occurring during periods of high atmospheric exposure to that fungus, and in whom pharmacotherapy or avoidance is inadequate to control symptoms.<sup>57</sup> Furthermore, the review recommended that mold mixes should be avoided.<sup>57</sup>

**Extract mixes.** It is a common practice to use mixes of unrelated allergen extracts (such as a tree mix, weed mix, or mold mix) in formulating allergen treatment sets.<sup>58</sup> Considering that immunotherapy is effective only if relative high doses of each allergen extract are administered, the obvious concern with use of mixes is that the extracts in the mix to which the patient is not allergic will dilute those allergens responsible for their symptoms to below the level of clinical effectiveness. There is no intellectual

**TABLE V.** Alternatives to current SCIT

<b>Using currently available extracts</b>
Delayed absorption
Adjuvants
Reduced levels of IgE
Alternative routes of administration
Nasal/bronchial
Oral (including microencapsulation)
Sublingual
<b>Using modified extracts</b>
Allergoids/polymerization
Site-directed mutagenesis and deletions
Allergenic peptides
Immunostimulatory sequence-ODN
Fc $\gamma$ -allergen fusion proteins

justification for using mixes that contain noncross-reacting allergens to prepare treatment sets. Mixes of cross-reacting allergens, such as within a weed family or a grass tribe, on the other hand, are easily justified.

**Proteases.** All fungal extracts and some arthropod extracts contain proteases that are capable of disrupting the integrity of protein allergens within their own or other allergen extracts (Table IV).<sup>59</sup> With allergen extracts containing potent proteases, it is best to use glycerinated extracts to inhibit, as much as possible, autodigestion of allergens within the extract.<sup>21,57</sup> There appears to be differences in susceptibility, with grass pollen extracts being very susceptible and ragweed extracts relatively resistant to the effects of proteases.<sup>60</sup> Nevertheless, in mixing allergen extracts, the best policy is to not mix extracts of pollens, house dust mites, or animal danders with those that have protease activity. It is probably all right to mix protease-containing extracts with each other, because the susceptible allergens have already undergone autodigestion.<sup>60</sup>

## ALTERNATIVES TO CURRENT SCIT

### Alternatives using currently available allergen extracts

**Adjuvants and carriers.** The alternatives to the current practice of SCIT that use available allergen extracts are listed in Table V. Of those approaches, the 1 commonly used is adsorption to alum to reduce the rate of dissemination from the injection site and thus to reduce systemic reactions. Although frequently used in Europe, alum adsorbed extracts are available for only a limited number of allergens in the United States and are notably lacking for house dust mites and animal danders. Other substances have been used to retard systemic uptake, including L-tyrosine<sup>61</sup> and encapsulation in liposomes.<sup>62</sup> Another approach is to provide T<sub>H</sub>1 adjuvant effects as with monophosphoryl lipid A.<sup>61</sup>

**Reduced levels of IgE.** Reduction of the amount of specific IgE on mast cells should reduce the chance of reactions, thus increasing safety and allowing more rapid build-up to maintenance dosing. This has been demonstrated

using the monoclonal anti-IgE antibody, omalizumab, to facilitate the administration of rush immunotherapy to 159 patients with ragweed-induced allergic rhinitis.<sup>63</sup> Subjects who had received omalizumab for the preceding 9 weeks had a 5-fold reduction in the number of systemic reactions during a 1-day rush protocol, as well as a non-statistically significant reduction of reactions during subsequent weekly dose escalation from 10% of patients to none.

*Alternative routes of administration.* Allergen extracts can be administered intranasally to reduce symptoms on subsequent natural allergen exposure. It was determined that this could be performed without undue side effects by administering cromolyn sodium before the allergen extract. Although this approach enjoyed a period of some popularity, especially in Italy, it has now largely fallen from favor.<sup>64</sup> Oral administration of allergen extract was also demonstrated to be effective, but gastrointestinal side effects contributed to its lack of popularity.<sup>64</sup> As opposed to the declining interest in intranasal and oral immunotherapy, there is currently great interest and widespread use in Europe of sublingual immunotherapy (SLIT).<sup>64</sup>

## SLIT

### Established aspects

*Diseases/conditions.* Meta-analyses have confirmed that SLIT is effective therapy for allergic rhinitis,<sup>65</sup> including in pediatric patients,<sup>66</sup> and for allergic asthma.<sup>67</sup> A meta-analysis of 21 trials involving 959 subjects with allergic rhinitis found a significant reduction in symptoms and medication use, but no clear relationship between results and the dose or duration of therapy could be established.<sup>65</sup> The pediatric rhinitis study, which included trials with 484 subjects, found significant improvement in symptoms with pollen, but not house dust mite extracts, and with treatment for more than 18 months, but not with briefer treatment.<sup>65</sup> Twenty-five studies with 1706 participants were included in the asthma meta-analysis.<sup>67</sup> Although the evidence found in the meta-analysis was not very strong, the results from assessing all parameters suggested SLIT reduced asthma expression.

*Safety/convenience.* The major advantage offered by SLIT over SCIT is safety; no fatal or near fatal reactions have been reported.<sup>68</sup> This reputation for safety has allowed home administration of SLIT, thus avoiding the other shortcoming of SCIT, the inconvenience of frequent visits to a physician's office to receive the injections. The absence of fatal or life-threatening reactions with SLIT does not, however, mean that this form of treatment is without adverse reactions. The predominant reaction is oral pruritus or swelling. In a double-blind study, 634 subjects with grass allergy received an oral tablet of timothy extract containing 15 µg Phl p 5 without a build-up phase. Oral itching or swelling was reported by 46% receiving active and 4% receiving placebo therapy.<sup>69</sup> A review of all published studies of SLIT from 1986 to 2004 included 25 studies, approximately half in which the dose was 1 to 50 times the customary SCIT dose and half in which the

dose was 50 to 500 times the SCIT dose.<sup>70</sup> The rate of systemic reactions including ocular, cutaneous, and respiratory was similar in the low-dose and high-dose studies, about 0.5 per 100 doses. In another review of the literature on SLIT, information on the occurrence of serious adverse reactions was available from studies including 1,019,826 doses administered to 3984 patients.<sup>68</sup> There were 14 probable SLIT-related serious adverse events, the majority being respiratory, with single episodes of uvula edema, urticaria, abdominal pain, and vomiting that resulted in hospitalization. One cutaneous-respiratory reaction to a multiple allergen mix has been reported.<sup>71</sup> Symptoms suggested hypotension, but a fall in blood pressure was not documented.

### Concerns and controversies

*Diseases/conditions.* The safety of administering extracts by SLIT compared with SCIT suggests the possibility of using this approach to immunotherapy for treating patients with allergy to foods. Success in individual patients with anaphylactic sensitivity to food has been reported.<sup>72</sup> A double-blind, placebo-controlled study of SLIT was undertaken in 22 patients with sensitivities to hazelnuts varying from the oral allergy syndrome to anaphylaxis.<sup>73</sup> After inpatient up-dosing over a period of 4 days, maintenance doses were administered at home for 4 months before subjects underwent a repeat oral challenge with hazelnuts. The mean threshold dose for provoking symptoms increased significantly in the active treatment group from 2.29 g before to 11.56 g after treatment, compared with 3.49 g and 4.14 g, respectively, in the placebo group.

There is also a preliminary report of a double-blind, placebo-controlled study of SLIT with house dust mite extract in children with atopic dermatitis.<sup>74</sup> Significant improvement occurred in children with mild but not in those with moderate or severe disease.

*Doses/frequency/dose response.* One of the unresolved questions with SLIT is that of dosing. The most common way to express the doses used in SLIT is to compare the cumulative amount administered sublingually over a period of 1 month to that administered by the same investigators for monthly maintenance SCIT. This has the limitation that maintenance doses used for SCIT may vary widely among investigators. With this limitation in mind, in a review of 48 randomized, mostly double-blind, trials with SLIT, it was found that doses over a 1000-fold range were reported to produce clinical improvement.<sup>68</sup> Furthermore, the rate of success did not increase as the dose increased. Symptoms or medication use improved in 72% of studies using cumulative doses 0.5 to 5 times those employed in SCIT, whereas for those using cumulative doses 6 to 50 times or greater than 50 times the SCIT dose, improvement in either of the 2 parameters was observed in only 55% of studies.<sup>68</sup>

It has been suggested that this apparently flat dose response is a result of limited absorptive capacity of the sublingual mucosa.<sup>75</sup> Very low doses (cumulative doses of 0.18-0.56 times the SCIT dose) were administered once,

twice, or three times daily for 1 year, after which all 3 groups received treatment 3 times a day for an additional year. During the first year, skin reactivity was reduced only in the 3 times daily group, whereas medication use was significantly reduced in both the 2 and 3 times daily groups. In the second year, when all received treatment 3 times daily, both skin test reactivity and medication use were reduced in all 3 groups compared with the controls. Other studies<sup>76-79</sup> (1 double-blind<sup>76</sup> and 3 open with controls<sup>77-79</sup>) using cumulative doses ranging from 0.5 to 2.5 times the SCIT dose reported significant improvement in symptoms,<sup>76-79</sup> medication use,<sup>77-79</sup> pulmonary function,<sup>76-78</sup> methacholine sensitivity,<sup>77,78</sup> nasal eosinophilia,<sup>77</sup> serum eosinophil cationic protein,<sup>76</sup> and IL-13.<sup>76</sup> In these same studies, there were significant reductions in the development of new sensitivities<sup>77</sup> and new asthma<sup>79</sup> and a reduction in existing asthma that persisted 5 years after the end of treatment.<sup>78</sup>

The only large, prospective assessment of a dose-response with SLIT was conducted with sublingual grass tablets in 3 doses (cumulative doses 0.75, 7.5, and 22.5 times the customary SCIT dose).<sup>80</sup> In this preseasonal study, only the highest dose produced a significant reduction in symptoms and medication use. Although this shows that a dose response can be demonstrated with SLIT, the period of administration was quite short compared with the studies in which lower doses have been effective.<sup>77-79</sup> In the dose-response study, the reduction in symptoms and medication in patients treated with the highest dose for at least 8 weeks before the pollen season was 21% and 29%, respectively.<sup>80</sup> When the same dose was used but administered at least 16 weeks before the pollen season, the reduction in symptoms was 30% and medication use, 38%.<sup>69</sup> Two doses of grass extract were tested in 71 children. The low dose was 80 and the high dose 300 times the customary SCIT dose.<sup>81</sup> Symptom/medication scores during the grass pollen season were significantly lower in the high-dose group, whereas side effects were comparable. The overall impression is that large doses may be more effective in short-term studies; however, the frequency and duration of treatment are important determinants of the response in SLIT.

**Multiple allergens.** Combinations of multiple allergens, as are commonly used with SCIT in the United States, have not been used in controlled studies of SLIT. If, indeed, the absorptive capacity is limited in the sublingual area, the use of multiple allergen mixes must be examined in controlled studies before widespread use of SLIT with multiple allergens can be endorsed.

**Efficacy relative to SCIT.** Two studies addressed the relative effectiveness of SLIT and SCIT.<sup>82,83</sup> Fifty-eight subjects with birch pollen allergic rhinitis underwent an observation year followed by randomization to receive placebo, SCIT with birch pollen extract at a relatively low monthly maintenance dose containing 3.28  $\mu$ g Bet v 1, or SLIT with a cumulative monthly dose 225 times the SCIT dose.<sup>82</sup> Only the first treatment season results were evaluable. Compared with placebo, symptom scores were reduced about twice as much by SCIT as by SLIT,

whereas medication use was reduced about 25% more with SCIT than with SLIT. Both treatments were significantly better than placebo but not significantly different from each other, perhaps because of the small number in each treatment group. The second study of SLIT did not have a direct comparison group on SCIT, but the authors had previously conducted a SCIT study in a similar group of grass-sensitive patients with allergic rhinitis.<sup>83</sup> They compared the degree of response to SCIT with that in the SLIT study that used a cumulative dose 45 times greater than SCIT. Reductions in symptoms and medication in the SCIT study were 60% and 80%, respectively, whereas in the SLIT study, the reductions were 28% and 45%, respectively, and not significantly different from placebo. The global improvement with SLIT was also about half that reported by the SCIT patients. Taken together, these studies suggest that a single preseasonal course of SLIT with a dose 45 to 225 times that given by SCIT will be about 1/2 as effective.

**Progression of sensitization and to asthma.** Studies show a significantly reduced development of new skin test reactions in monosensitized patients treated with SLIT compared with untreated controls.<sup>79</sup> In an open study, 97 children with allergic rhinitis and not more than 3 wheezing episodes during the previous grass pollen season were treated with either low-dose, preseasonal SLIT or symptomatic therapy for 3 years. There were no differences in the first year of treatment; however, symptoms the second year and medication use the second and third years were significantly less in the active treatment group. By the third season, 18 of the children in the observational group had developed seasonal asthma compared with only 8 of the treated children. This difference was statistically significant.

**Persistence of effect.** There are few long-term follow-up studies after discontinuation of SLIT. One open study in children showed a significant reduction in asthma after 5 years of therapy with house dust mite extract.<sup>78</sup> This reduction in prevalence of asthma persisted without change on follow-up 5 years after SLIT was discontinued.

**Compliance with SLIT.** Compliance and adherence to SLIT have been assessed.<sup>84,85</sup> Patients who had been prescribed unit dose SLIT were contacted during the 3rd and 6th months of therapy by unscheduled phone calls.<sup>84</sup> Patients were asked to count the remaining doses. Compliance in 443 patients in the 3rd month was >90% in 76% of patients, and in 226 patients in the 6th month was >90% in 75% of 226 patients. Overcall adherence to a 3-year course of immunotherapy was assessed in 2774 children in Southern Italy and Sicily.<sup>85</sup> The highest rate of discontinuance was nasal immunotherapy, 73.2%; next, SLIT, 21%; and least, SCIT, 10.9%. Major reasons for discontinuation were unpleasantness for nasal immunotherapy, ineffectiveness for SLIT, and inconvenience for SCIT.

### Alternative therapy with modified extracts

**Allergoids/polymerized extracts.** Creation of an allergoid by treatment with formaldehyde<sup>86</sup> and polymerization with glutaraldehyde<sup>61</sup> both reduce allergenicity of the extract while retaining immunogenicity. Research

continues with both approaches in Europe, but not in the United States.

*Recombinant allergens with site-directed mutagenesis and deletion.* Successful immunotherapy has been reported using a mixture of recombinant major allergens of grass.<sup>87</sup> Although this produces a consistent extract, there is no reduction in allergenicity and hence limited advantage over natural allergen extracts. Site-directed mutagenesis or deletion, on the other hand, can reduce the IgE epitopes on the molecule while not affecting its ability to react with T lymphocytes. This approach has been taken to human studies with fragments or trimers of the major allergen of birch, Bet v 1.<sup>88</sup>

*Allergen-derived peptides.* Because the IgE epitopes are expressed on the 3-dimensional structure of the allergen while the T lymphocyte epitopes are short segments of adjacent amino acids, it is possible to devise peptides too small to react with IgE but with retention of the T lymphocyte epitopes. Thus, reduced allergenicity with retained immunogenicity is achieved. This approach is being actively pursued with peptides of the major cat allergen, Fel d 1.<sup>89</sup>

*Immunostimulatory sequences.* Unmethylated cytosine phosphorothionate guanosine segments that characterize bacterial and viral DNA react with the Toll-like receptor 9 of antigen presenting cells.<sup>90</sup> Synthetic cytosine phosphorothionate guanosine DNA chains, when covalently linked to allergens, have the advantage not only of directing the response to the allergen toward a regulatory and T<sub>H</sub>1 bias but also of sterically interfering with the reaction of IgE with the allergen, thus reducing its allergenicity. In a study of 25 well characterized ragweed-sensitive patients, a single 6-injection course of treatment with immunostimulatory DNA and Amb a 1 (maximum dose, 12 µg Amb a 1) resulted in improvement in rhinitis symptoms for 2 years.<sup>90</sup> The symptoms each pollen season in the treated group were about 1/3 those in the placebo control. In a multicenter study of less carefully selected patients and geographical locations, 6 weekly injections of immunostimulatory sequence–Amb a 1 to a maximum dose of 30 µg resulted in a 21.2% reduction in symptoms.<sup>91</sup> The second ragweed season, without further treatment, symptoms were reduced 28.5%. Paradoxically, a group who received 2 booster injections before the 2nd ragweed pollen season did not fare as well; improvement was not significantly better than placebo.

*Fusion proteins.* Mast cells and basophils express FcγRIIb, which contains an immunoreceptor tyrosine-based inhibition motif within its cytoplasmic tail.<sup>92</sup> Aggregating FcγRIIb to the major IgE receptor, FcεRI, leads to inhibition of FcεRI signaling. A fusion protein consisting of human Fcγ plus the major cat allergen (Fel d 1) has been developed and tested as a new form of immunotherapy.<sup>92</sup> Pretreatment of basophils from subjects with cat allergy and cat allergen–sensitized cord blood–derived mast cells with the fusion protein reduced histamine release on Fel d 1 challenge.<sup>92</sup> Active immunization of mice with the fusion protein produced inhibition of systemic, lung, and cutaneous reactivity to Fel d 1.<sup>93</sup>

## CONCLUSION

Conventional SCIT is unquestionably effective for *Hymenoptera* venom sensitivity and for the allergic component of rhinitis and bronchial asthma. Effective doses have been defined for many of the common allergens.

Immunotherapy remains the only truly disease-modifying treatment for asthma and allergic rhinitis. It restores the normal immune response to common allergens. It can prevent new sensitizations in monosensitized individuals and prevent the development of clinical asthma in those with only rhinitis. Furthermore, its salutary effects continue for years after completion of the course of treatment. The factors limiting the greater use of immunotherapy are concerns of safety and the inconvenience of prolonged build-up and maintenance treatment schedules. Numerous approaches to improve the safety and increase the convenience of immunotherapy are being studied. The only one currently in clinical use is sublingual administration of the allergen extracts. Evidence for the effectiveness of this approach is quite convincing. There remain questions to be resolved including optimal dosing, frequency, and duration of treatment. There is also the question of the effectiveness of administration of mixes of multiple allergen extracts sublingually. Finally, SLIT appears to be, at least in the short term, less effective than SCIT. It is likely there is a place for both approaches to immunotherapy in the treatment armamentarium.

Other new approaches to immunotherapy are under study. These include modified recombinant allergens, peptides, immunostimulatory DNA sequences covalently bound to allergenic proteins, and fusion proteins designed to give an inhibitory signal to mast cells and basophils. Whether any of these approaches will replace current immunotherapy practices depends on the demonstration of increased safety and convenience, cost effectiveness, and retention of the efficacy of current injection immunotherapy.

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