

Allergy immunotherapy: Future directions for the 2020s

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ABSTRACT

Allergy immunotherapy (AIT), whether administered as subcutaneous immunotherapy or as sublingual immunotherapy (SLIT), is an effective treatment for sensitization to inhalant allergens. There remain, however, some important unresolved issues, such as the need for compelling evidence for or against the efficacy of treatment with multiple unrelated allergen extracts and optimal dosing with SLIT-liquid preparations. Both methods of AIT involve prolonged periods of treatment to achieve persisting benefit. This can be inconvenient and expensive, and failure to complete the period of prescribed treatment is common with both methods. New approaches are being developed and studied to make AIT more effective, safer, or more convenient. Among these approaches are using alternative routes of administration; using adjuvants, including vitamin D, Toll-like receptor ligand agonists, biologics, or probiotics; introducing additional SLIT tablets; defining the patterns of major and minor allergen sensitivity of patients and the content of allergen extracts to better match sensitization with treatment; and treating cats to reduce their allergen release. The allergen molecules themselves are being altered to make them less reactive with specific immunoglobulin E, both by creating allergoids and by using recombinant technology to produce modified allergen molecules. Which, if any, of these new approaches will become part of AIT practice in the next decade depends in part on their efficacy and in part on the availability of the resources to adequately study them.

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Allergy immunotherapy (AIT) was introduced by Drs. Noon and Freeman in 1911 as a means of inoculating patients with hay fever against a toxin in grass pollen to which the patients were uniquely sensitive.¹ Although there are similarities in the methods that were used by Drs. Noon and Freeman and those used by allergists today, there have also been many changes in practice and concept. Within a few years of the introduction of AIT, the practice had been widely embraced and the treatment was used, not only for pollen-induced allergic rhinitis but also for perennial rhinitis and bronchial asthma.¹

The presence of a sensitizing constituent in the blood, reagin, later identified as immunoglobulin E (IgE), rather than a toxin in grass pollen as the responsible agent was demonstrated by Drs. Prustnitz and Koestner,¹ and the strong familial tendency to develop these “atopic” diseases was recognized by Drs. Coca and Cooke.¹ The initial approach to AIT was by subcutaneous injections of increasing amounts of pollen extract (subcutaneous immunotherapy [SCIT]). In the past few decades, administration of the allergen extract by the sublingual route (sublingual immunotherapy [SLIT]) has been shown to be effective, and, in some countries, this has become the dominant approach to

administration. The efficacy of SCIT with Hymenoptera venom as treatment for Hymenoptera sensitization was demonstrated²; some evidence demonstrated efficacy of AIT in selected patients with atopic dermatitis who were sensitized to house-dust mites (HDM).³ Advances have been made in understanding the immune mechanisms that underlie the efficacy of AIT, first, the demonstration of a specific blocking antibody and, later, the generation of allergen-specific regulatory T (Treg) cells and allergen-specific immune deviation from a type 2 to a type 1 CD4⁺ T-helper (Th) cell response.¹

Despite these improvements in the clinical application and immunologic understanding of AIT, there remain problems that limit its use. Some problems, *e.g.*, cost, are beyond the scope of this review. However, other problems that relate to matters of safety, the inconvenience of multiple visits to a clinic to receive subcutaneous injections, and the 3- to 5-year duration of both SCIT and SLIT are amenable to improvement. In addition, some aspects of the current practice of AIT lack definitive supporting scientific evidence. Notably, this applies to the use of mixtures of multiple unrelated allergen extracts both for SCIT and for SLIT. Also, there is a need for more studies that define optimal dosing, particularly for SLIT, with the liquid extracts that are being used off label in the United States.⁴ This review will address those improvements in AIT that seem to be achievable within the next decade.

FUTURE DIRECTIONS FOR AIT, SUPPORTING STUDIES FOR THE CURRENT PRACTICE OF AIT

Future directions for AIT are discussed in Table 1.

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Table 1 Potential future strategies to improve the safety and/or efficacy of AIT

1. Establish proof of the efficacy or lack thereof of multiallergen SCIT and SLIT, and determine optimal doses for SLIT-liquid preparations
2. Change to a more efficient route of administration, such as intralymphatic or epicutaneous
3. Use adjuvants, such as probiotics or vitamin D
4. Co-administer with Th1- or Treg-promoting TLR ligands, such as CpG oligonucleotides, monophosphoryl lipid A, or a CRL ligand, *e.g.*, mannan
5. Identify patterns of major allergen sensitization in patients and major and minor allergen content of allergen extracts to provide "precision medicine"
6. Increase the dose of allergen and shorten the course of treatment by reducing allergenicity by chemical modification (*e.g.*, allergoids) or recombinant modification, such as modified allergens or non-IgE binding peptides
7. Use nanoparticles to bypass specific IgE and target the immune system

AIT = Allergy immunotherapy; SCIT = subcutaneous immunotherapy; SLIT = sublingual immunotherapy; Th1 = T-helper type 1; Treg = T regulatory; TLR = toll-like receptor agonist; CpG = cytosine phosphodiester guanine; CRL = C-type lectin receptor; IgE = immunoglobulin E.

Multiallergen AIT

There are significant uncertainties with regard to the use of both SCIT and SLIT that need to be addressed. In particular, there is a need to resolve the question of the efficacy of administering mixtures of multiple unrelated allergens. A major difference in AIT between the United States and Europe is the approach to the patient who is multiply sensitized. In Europe, the recommended practice is to treat with only the one or possibly the two allergen extracts that are considered clinically most important to the patient.⁵ In the United States, however, the common SCIT practice, supported by the American Academy of Allergy, Asthma & Immunology/American College of Allergy, Asthma, and Immunology Immunotherapy Practice Parameters third update⁶ is to include all clinically relevant allergens.

The studies that support the U.S. practice have been reviewed.⁷ The primary support is provided by three studies;⁷ two studies by Drs. Lowell & Franklin were in patients with ragweed-induced allergic rhinitis.⁷ In these studies, the patients were paired based on their symptoms during the previous ragweed pollen season. Before the next pollen season, ragweed extract was removed or reduced by 95% in the treatment of one of each pair. The removal or reduction in ragweed content significantly increased symptom and medication scores during multiple weeks of the ensuing ragweed

pollen season. Because, in each patient, the ragweed was part of a multi-allergen mixture being administered by SCIT, the studies demonstrated the effectiveness of an individual pollen extract in a mixture of unrelated extracts. The third study,⁷ by Dr Michael Reid and co-authors was similarly designed, except that the patients did not undergo an observational season and grass pollen extract was the component of the patients' multi-allergen treatment extract that was either included or substituted with placebo.⁷ In this study, asthma symptoms were significantly reduced during the ensuing grass pollen season, whereas symptoms of allergic rhinitis also trended to improvement. These studies are criticized because the number of subjects is small, but they were carefully conducted and the consistency and level of significance of the findings is impressive.

The evidence for the use of multiple-allergen mixes in SLIT is even more limited. There are two studies that indicate that two unrelated allergen extracts can be co-administered sublingually with clinical efficacy.^{8,9} In one of these studies, the results with the two allergen extracts were found to be comparable with those when each of the extracts were being administered as monotherapy.⁸ There is only one study, to my knowledge, that examined the efficacy of co-administration, sublingually, of more than two unrelated extracts.¹⁰ In that study, SLIT with 1 mL of concentrated timothy extract diluted to 10 mL with saline solution was compared with the same dose of timothy diluted to 10 mL by the addition of nine unrelated pollen extracts and to a placebo, which consisted of saline solution colored by caramelized sugar.¹⁰ In the participants who received 9 months of SLIT with timothy monotherapy compared with those who received placebo, thresholds for specific titrated nasal challenge, titrated skin-prick tests (SPT), and levels of serum specific IgG4 (sIgG4) were increased ($p=0.03$, $p=0.001$, and $p=0.005$, respectively), whereas, in those who received the timothy in the same dose and dilution mixed with unrelated pollen extracts, there was significant improvement versus placebo only in the titrated SPTs ($p=0.04$).¹⁰ This study suggests that there may be a problem with multiple-allergen SLIT that could relate to transport of the allergens through the sublingual mucosa or to phagocytosis and transport of the allergen by the sublingual dendritic cells (DC's).¹⁰

SLIT Dosing with Liquid Extracts

Double-blind, placebo controlled studies have established doses for a number of pollens and indoor allergens that are effective and sometimes those that are ineffective when administered by SCIT.¹¹ Dosing with SLIT tablets has been carefully studied in large studies with two or more doses being compared with placebo.¹²⁻¹⁵ There are properly controlled studies with

Table 2 Double-blind, placebo controlled studies that demonstrate efficacy with SLIT-liquid extracts

Study, y	Allergen	Dose (multiple of recommended SCIT monthly dose*)	Duration of Study	Outcome of Study
Wahn <i>et al.</i> , ¹⁶ 2012	Grass	48 µg, group 5 daily (×48)	Pre- and coseasonal, 1 y	Symptom and medication score reduced 21% vs placebo
Creticos <i>et al.</i> , ¹⁷ 2014	Ragweed	50 µg Amb a 1 (×125)	Pre- and coseasonal 1 y	Symptom and medication score reduced 20% during peak of season
Wang <i>et al.</i> , ¹⁸ 2014	House-dust mites	28 µg Der p 1 + 50 µg g Der f 1 (×275)	1 y	ACQ improved, ICS dose reduced
Pfaar <i>et al.</i> , ¹⁹ 2019	Birch	100 µg/d (×214)	3–6 mo pre-seasonal + season	Symptom and medication score was reduced 32% compared with placebo

SLIT = Sublingual immunotherapy; SCIT = subcutaneous immunotherapy; ACQ = Asthma Control Questionnaire; ICS = Inhaled Corticosteroids.

*From Ref. 6.

SLIT-liquid preparations that demonstrate efficacy but rarely have multiple doses been compared in the same studies (Table 2). In the cited studies,^{16–19} the cumulative monthly dose of SLIT liquid ranged from 48 to 275 times the recommended monthly SCIT maintenance dose with the same allergen.⁶

The uncertainty with regard to appropriate SLIT-liquid dosing is also demonstrated by the wide range of SLIT-liquid doses being offered commercially by extract companies in Europe.²⁰ Again, when the cumulative monthly doses of SLIT (Allergy Therapeutics, HAL, and Stallergenes) liquid from these three European extract companies were compared with U.S.-recommended SCIT maintenance doses,⁶ the range of ratios for HDM was 1 to 16 times, for cat dander was 1 to 31 times, for timothy was 21 to 94 times, and for ragweed was 42 to 237 times the SCIT monthly dose. The uncertainty of appropriate SLIT-liquid dosing is also apparent in the most-recent report on SLIT practices in the United States.⁴ American allergists, who have been using the U.S. Food and Drug Administration (FDA) approved liquid extracts “off label” for SLIT have been using monthly cumulative doses that, in relation to monthly dosing with SCIT, vary from <1 to 50–100 times. Approximately two-thirds of the U.S. allergists who reported using SLIT liquid used doses less than those that have been shown necessary for optimal treatment with SLIT tablets.

Implication. The results of studies of multi-allergen SCIT would be unlikely to change the practice of SCIT in the United States, but, if supportive of the U.S. practice, then might bring European practices closer to the current U.S. practices. Determination of the efficacy of multi-allergen SLIT, however, has important implications for the future

use of SLIT in the United States, where treatment is rarely limited to one or two allergens. If multi-allergen SLIT is relatively ineffective, then it will favor SCIT as the dominant method of AIT in the United States. Determination of optimal dosing for SLIT liquids would ensure, if they are used appropriately, that patients do not invest time and money in ineffective therapy.

ALTERNATIVE AND COMPLEMENTARY APPROACHES TO AIT

There are a number of alternative or complementary approaches to AIT, many of which seem almost ready for clinical application; these include (1) administration of extracts by alternative routes, such as injection into lymph nodes or application epicutaneously with patches; (2) use of adjuvants, such as vitamin D, TLR-agonists, biologics, or probiotics, to enhance the effect of AIT; (3) introduction of additional SLIT tablets, such as birch and Japanese red cedar; (4) use of allergens or allergen-specific antibodies to determine patients' patterns of sensitivity to major allergens and analysis of the major allergen content in those extracts that contain multiple major allergens such as dog and cockroach; and, finally, (5) treating cats, either actively or passively, to reduce their release of the major allergen, Fel d 1.

Alternative Routes of Allergen Administration

The efficacy and safety of AIT can potentially be improved by administering the allergen extract into tissues that have a high density of antigen-presenting cells and a low density of mast cells and vascularity.²¹ These conditions are met by both intralymphatic immunotherapy (ILIT) and epicutaneous immunotherapy (EPIT) administration.

ILIT. In a large, open trial, ILIT with three injections of timothy extract at 4-week intervals was compared with a conventional 3-year course of timothy SCIT.²² The ILIT was well tolerated and induced fewer systemic reactions than SCIT. After 4 months, ILIT but not SCIT, improved the threshold of nasal allergen challenge, and, after 3 years, there was no difference in nasal allergen challenge or symptoms during the grass pollen season between the two forms of AIT. Subsequently, several small studies have supported these favorable results,²³ but a double-blind, placebo controlled study in which ILIT was administered at 2- rather than 4-week intervals found no difference between active and placebo treatment.²⁴ Therefore, a final decision on ILIT awaits reporting of a large controlled study that duplicates the methods and results of the original study.

EPIT. The same Swiss group that conducted the original study with ILIT, reported the results of three studies of EPIT.²⁵ In these three studies, all with grass pollen extract, the investigators examined the number of weekly patches applied after repeated tape stripping of the skin, the duration the patches remain on the skin, and the dose of allergen applied.²⁵ In the final study,²⁵ in which they applied six weekly grass pollen extract patches, each remaining on the skin for 8 hours, they reported a 48% and 40% reduction in allergic rhinitis symptoms in the first and second grass pollen season without further treatment compared with 10% and 15% reductions for placebo. Adverse reactions seem to be acceptable, with localized eczema, after 18% of the patches, and 4% of the subjects experienced mild systemic symptoms.

Adjuvants

Adjuvants administered in conjunction with AIT act on the immune system to enhance the response to the administered allergens. Four adjuvants are currently used in products marketed in Europe: aluminum hydroxide, microcrystalline tyrosine, monophosphoryl lipid A, and calcium phosphate, all of which are thought to promote tolerance. Other adjuvants, such as probiotics and vitamin D, are readily available but their efficacy requires further studies, whereas the TLR-agonists and related molecules and biologic agents continue to be investigated.

Vitamin D and Probiotics. Both vitamin D and probiotics have been shown to affect the immune system, including the promotion of the development of Treg cells. The effect of serum levels of 25-hydroxy Vitamin D at baseline and after 3 months on the response to 12 months of SCIT with HDM extract was assessed in 36 children, ages 6–12 years.²⁶ Children with a higher serum level of 25(OH)D experienced a more significant

reduction in asthma symptom scores and inhaled steroid dose, and had higher Transforming growth factor (TGF) production and higher Forkhead box P 3 (Fox p 3) induction during AIT. This was especially true in those with a serum level >30 ng/mL. In a nonrandomized trial, 48 patients with allergic rhinitis due to date palm pollen and who also had vitamin D deficiency were treated with either SCIT to date palm pollen alone or with added vitamin D 50,000 IU weekly for 3 months, then 2000 IU daily for 3 months.²⁷ After 6 months, the group that was receiving supplementary vitamin D had a significantly greater reduction in nasal symptoms and elevation of serum interleukin (IL) 10 ($p < 0.001$ for both), with a significant inverse correlation between the two. In China, 80 patients with perennial allergic rhinitis, HDM sensitivity, and serum vitamin D₃ levels that averaged 20 to 30 ng/mL were randomized to placebo, HDM SCIT, vitamin D 2000 IU/day, or the combination of SCIT and vitamin D for 6 months.²⁸ Both SCIT and vitamin D alone significantly reduced sIgE levels, SPT results, serum IL-13 levels, and symptom and medication scores compared with placebo. The combination reduced all these outcomes further, compared with each treatment alone.

Fifty children, ages 5–12 years, participated in a 5-month trial in which they received a 300 IR SLIT 5-grass tablet daily plus vitamin D 1000 IU daily or placebo.²⁹ Children who received vitamin D fared significantly better for nasal and asthma symptoms. The same group also reported on a larger trial of 100 children, perhaps including the group reported in reference 29.³⁰ Children received the five-grass SLIT tablet for 5 months combined with either vitamin D 1000 IU, a probiotic, *Lactobacillus rhamnosus* GG, or placebo.³⁰ There also was an untreated allergic rhinitis control group. The investigators reported a reduction in the symptom and medication score compared with the control group, of 50% in SLIT-placebo, 67% in SLIT-vitamin D, and 80% in SLIT-probiotic.³⁰ There was also improvement in lung function and a significant increase in the percentage of DC4⁺CD25⁺FoxP3⁺ T cells (Treg) in children who received grass SLIT.³⁰ The children treated with SLIT and probiotic had significantly greater induction of the Tregs compared with the SLIT-placebo group.³⁰

Very impressive results with the addition of a probiotic to SCIT have been reported from China.³¹ Patients with perennial allergic rhinitis, monosensitized to HDMs, were randomized to placebo, SCIT with HDM extract, oral *Clostridium butyricum* twice daily, or SCIT plus the probiotic for 6 months, with 6-month post-treatment follow-up.³¹ There were no responses with placebo or probiotic alone, there was transient improvement in nasal symptom and medication scores with SCIT alone, but greater and sustained improvements in nasal symptom and medication scores and in

sIgG4 levels, serum IL-4 levels, and SPT results were observed with SCIT combined with the probiotic.³¹

TLR and Related Molecules. The TLRs of the innate immune system recognize molecular patterns common to bacteria and viruses and, when stimulated, release a number of interferons and interleukins that favor Th1 and Treg cell responses. Studies were conducted with unmethylated type B cytosine phosphodiester guanine CpG DNA motifs, which stimulate TLR-9, combined with the major allergen of ragweed, Amb a 1, but, after initially encouraging results,³² the failure to replicate them in large studies led to abandonment of the product.³³ Studies were also conducted with a type A unmethylated CpG motif, protected from DNases in a virus-like particle, without any specific allergen.³⁴ Again, initial studies were encouraging, but the product was abandoned after failure in later trials.³⁵ A derivative of the cell wall of *Salmonella minnesota*, monophosphoryl lipid A (MPL), a ligand for TLR-4, is incorporated into a European product that requires only a few injections. However, a trial with MPL and a grass allergoid in the United States produced disappointing results.³⁶ Currently, the dosing regimens with this product are being reexamined.³⁷ *In vitro* studies have shown that polymerized allergoids coupled to nonoxidized mannan are recognized by C-type lectin receptors and better endocytosed by DCs than are allergoids or native allergens alone, this favors a Th1/Treg cell response.³⁸ In early human studies, compared with the allergoid alone; the allergoid-mannan product showed hypoallergenicity, was captured more efficiently by human DCs, induced greater IL-6 and IL-10 release from DCs, and promoted the generation of functional FoxP3⁺ Treg cells.³⁸

Lysosomal-Associated Membrane Protein-1. Encoding allergen DNA in a viral protein that is then linked to lysosomal-associated membrane protein-1 (LAMP) results in selective routing to the endosomal and lysosomal compartments of DCs, which leads to major histocompatibility complex class II processing and a strong Th1 response.³⁹ A plasmid was constructed that contained Cry J2 allergen from Japanese red cedar and LAMP. In a phase I study to assess the safety of the product, observations were made on the subjects' SPT results. Of 12 subjects with positive SPT results to Japanese red cedar, 10 had negative results after four intramuscular injections at 2-week intervals, whereas 6 of 11 with positive SPT results to mountain cedar also converted to negative.

Biologics. The monoclonal anti-IgE antibody, omalizumab, has been used as an adjuvant in AIT, primarily to reduce levels of serum IgE and reduce systemic reactions to the injected allergen. This strategy has

been successfully used in rush SCIT with ragweed in patients with allergic rhinitis⁴⁰ and in cluster therapy in patients with asthma due to HDM, cat, or dog sensitization.⁴¹ As far as is known, omalizumab does not affect the immune response to AIT.

Two studies^{42,43} were conducted with biologics that are directed at the type-2 immune response, with the expectation of enhancing the response to AIT. Thirty-seven subjects with grass allergy received a suboptimal course of grass pollen SCIT with or without four monthly doses of a monoclonal anti-IL-4, or matching placebo.⁴² During the grass pollen season, both of the actively treated groups had significantly reduced symptoms compared with placebo, but there was no difference between the two treatment groups for symptoms or immunologic outcomes.⁴² In an Immune Tolerance Network study,⁴³ 121 subjects with cat allergy were randomized to receive cat SCIT, the anti-TSLP monoclonal antibody tezepelumab, both SCIT and antibody, or double placebo. The subjects received treatment for 1 year, then were followed up for an additional year.⁴³ The primary outcome was titrated nasal allergen challenge with cat hair and dander extract. The study is completed but results have not been reported.⁴³

Additional SLIT Tablets

There is current FDA approval for SLIT tablets for timothy, 5-grasses, short ragweed, and mixed *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*. SLIT tablets prepared from birch pollen⁴⁴ and Japanese cedar pollen extracts⁴⁵ are undergoing registration studies in Europe and Japan, respectively. Based on the amino-acid sequence homology of the major allergens and IgE cross-reactivity, five tree species (birch, alder, hornbeam, hazel, and oak) have been assigned to the birch homologous group.⁴⁶ The birch SLIT tablet, which contains 60 µg of Bet v 1 in its 12-DU formulation, was administered to 634 adolescents and adults with birch allergy and their symptom and/or medication scores (TCS) were monitored during the ensuing sequential hazel, alder, and birch pollen seasons.⁴⁴ The Total Cumulative Score was reduced 40% compared with placebo, during the birch pollen season and 37% during the three-tree season.⁴⁴

The same tablet was studied by using an environmental exposure chamber (EEC) in which symptoms on exposure to both birch and oak, separately, were evaluated before and after 24 weeks of treatment.⁴⁶ The 12-DU birch SLIT tablet reduced the total symptom score compared with placebo, 25% on exposure to birch pollen and 24% on exposure to oak pollen,⁴⁶ which suggests a strong crossover effect; however, the results could be influenced by the patients' primary sensitization. If it was to oak pollen, then the results are valid; however, if their primary

sensitization was to birch, then the results may overstate the degree of cross-efficacy.

Members of the family Cupressaceae are recognized as being highly cross-reactive. Mountain cedar pollen is recognized as a major cause of allergic symptoms in Texas, and ornamental plantings of related trees and bushes cause pollen symptoms widely throughout the United States. A dose finding and long-term efficacy trial of a Japanese red cedar SLIT tablet was conducted in 1042 children and adults with allergic rhinitis.⁴⁵ In the first pollen season, the total nasal symptom and medication scores were reduced by 32.1% versus placebo by the dose selected for further studies (5000 Japanese allergy units); after 3 years of treatment, the reduction in the total nasal symptom and medication score versus placebo was 45.9%.⁴⁵

Implications. Of the alternative routes of administering AIT, the epicutaneous approach seems most promising, but other allergens besides grass need to be studied. The co-administration of vitamin D seems promising, at least in patients with reduced serum levels. Probiotics may be even more effective, but any results will apply only to the specimen of the strain that was studied. Of the SLIT tablets that are being developed, the Japanese red cedar might make a big impact in Texas, where mountain cedar is such a dominating aeroallergen. The birch tablet would be a welcome addition, if the degree of crossover protection for oak were as great as the one study⁴⁶ would suggest. The study should be repeated, however, with patients whose primary sensitization was by oak pollen. Results of studies with the TLR-agonists, CpG motifs, and MPL have been disappointing, but this may have been due to flaws in the study designs. The use of mannan or LAMP to enhance uptake by DCs and favor Th1/Treg development seems promising but is very early in development.

USE OF RECOMBINANT ANTIBODIES TO COMPONENT INDIVIDUAL ALLERGENS TO IDENTIFY PATIENTS' PATTERNS OF SENSITIZATION AND MAJOR ALLERGEN CONTENT OF ALLERGEN PRODUCTS

Recombinant proteins have been synthesized that represent major and minor allergens of many important pollen and environmental sensitizers. Their role in making a precise allergy diagnosis in patients has not yet been defined and probably will vary with sensitization to those sources with a dominant major allergen versus to more complex sensitizers. For those sources that contain one dominant major allergen, testing the patient toward that major allergen will offer little advantage over testing with the whole allergen extract, except, perhaps to alert the physician to sensitization to cross-reactive components such as profilins, polcalcins, and lipid

transfer proteins, which are shared by nonrelated plants. With more-complex allergen extracts that have several major allergens or no immunodominant allergen, determination of a patient's pattern of sensitization may be useful but only if information is also available on the content of these allergens in the allergen extracts available for treatment. An example of an extract with one dominant major allergen is birch pollen extract (Bet v 1).⁴⁷ Examples of allergen extracts with multiple major allergens and varying patterns of patient sensitization are cat, dog, and cockroach.⁴⁸

Patterns of Sensitization of Patients to Component Allergens

Representative patterns of sensitization of patients to component allergens are presented in Table 3. A major allergen is generally regarded as one to which >50% of patients with allergy react.⁴⁸ However, no major allergen has been shown to be positive in 100% of the subjects clinically allergic to that pollen or environmental substance. The major allergen that perhaps comes the closest to this goal is Bet v 1 of birch. More than 95% of patients allergic to birch have sIgE to Bet v 1, and, in 60%, it is the only birch allergen to which they are sensitized.⁴⁹ Evidence of the dominant role for Bet v 1 in subjects with birch allergy comes from a study that compared the response in nasal symptom and medication scores with SCIT with placebo compared with recombinant Bet v 1, natural Bet v 1, or birch pollen extract, all at a Bet v 1 dose of 15 µg at maintenance.⁴⁷ During both the first and second birch pollen season, there was a highly significant reduction in symptom and medication scores with all three active treatments and no difference in the response to Bet v 1 alone compared with the intact birch pollen extract.⁴⁷

Short ragweed has a dominant major allergen, Amb a 1, with >90% of patients with ragweed allergy having detectable levels of sIgE.⁵⁰ However, among the 10 other allergens to which patients who were ragweed sensitive react, Amb a 11 showed sensitization rates up to 66%, and, in 92 American, and European patients 15% were mainly sensitized to Amb a 11, which indicated that Amb a 11 should be included for the diagnosis and treatment of ragweed allergy.⁵⁰ Even greater heterogeneity of sensitization has been reported with timothy grass.⁵¹ In timothy grass pollen extract, there are a number of major allergens to which ~90% of subjects with timothy allergy show sensitization to Phl p 1, up to 85% to Phl p 5, and >50% to Phl p 2, 4, and 6. The ratio of allergen sIgE to total timothy IgE was highest for Phl p 1, followed by Phl p 5, and <10% for each of the other major allergens, which indicated that group 1 and group 5 are most important for most patients. However, the presence of a positive nasal and conjunctival allergen

Table 3 Major and important minor allergens in allergy extracts

Allergen Extract	Major* and important Minor# Allergens	Reported Rate of Sensitization in Subjects with Allergy, %
Birch pollen extract§	Bet v 1*	>95
Short ragweed extract¶	Amb a 1*	>90
	Amb a 11*	≤66
Timothy grass	Phl p 1*	90
	Phl p 5*	85
	Phl p 2*	>50
	Phl p 4*	>50
	Phl p 6*	>50
Cat hair and dander**	Fel d 1*	70–85
	Fel d 4#	19
	Fel d 7#	10
	Fel d 8#	15
Dog hair and dander##	Can d 1*	50–70
	Can f 4*	46–81
	Can f 5*	≤70
House-dust mites§§	Group 1*	73
	Group 2*	87
	Group 23*	47
	Groups 4, 5, 7, 13, 15, and 21#	>20 < 50
German cockroach¶¶	Bla g 1#	26–40
	Bla g 2*	54–72
	Bla g 4#	35
	Bla g 5#	39
	Bla g 6#	44
	Per a 7#	22
	Bla g 9#	44
	Bla g 11#	73

*Major allergens.

#Important minor allergens.

§From Ref. 49.

¶From Ref. 50.

||From Ref. 51.

**From Ref. 52.

##From Ref. 53.

§§From Ref. 56.

¶¶From Ref. 58.

challenge increased with an increasing number of timothy allergens to which the subjects were sensitized.

Compared with pollen extracts, those derived from animal and insect material tend to be more complex. Patterns of IgE binding to eight allergens was studied in 96 individuals allergic to cats.⁵² When using >0.35 IU/mL as a cutoff, 79% of the patients had sIgE to cat dander, 50% of the total group had sIgE to Fel d 1, whereas sIgE was also found to Fel d 4 in 19%, Fel d 7 in 10%, and Fel d 8 in 15%.⁵² More than 50% of the cat sIgE was bound by Fel d 1 in 60% of the patients, but the range of binding was 0 to 100%; in 25% of

patients, the non-Fel d 1 antigens bound >50% of the cat sIgE, which indicated that, in addition to Fel d 1, Fel d 4, 7, and 8 should be included in the diagnosis and treatment.⁵² Sensitization to the major allergens of dogs includes Can f 1 in 50–70%, Can f 4 in 46–81%, and Can f 5 in up to 70% of the subjects with dog allergy.⁵³ Positive nasal allergen challenge was associated with sensitization to an increasing number of dog allergens.⁵⁴ A study conducted in west Sweden, in which a limited number of dog and cat allergens were analyzed in an asthma population, found that 21% of subjects sensitized to Fel d 2, Fel d

4, Can f 3, or Can f 5 were not sensitized to the major allergens Fel d 1 or Can f 1.⁵⁵

An assessment of the pattern of sensitization to the HDMs *D. pteronyssinus* and *D. farinae* was conducted on the serum of 853 adults and 423 children from Europe and North America.⁵⁶ Sensitization to the group 1 allergens was found in 73% and sensitization to group 2 allergens was found in 87% of the subjects.⁵⁶ In addition, sensitization to group 4, 5, 7, 13, 15, 21, and 23 allergens was found in 20–47% of subjects.⁵⁶ Of particular note are group 23 allergens that caused sensitization in 47% of the subjects and have been suggested as major allergens but less important than groups 1 and 2.⁵⁶ Some of these sensitizing allergens are found in mite bodies, whereas others are found in mite feces. The investigators concluded that a mite allergen extract should contain both species of *Dermatophagoides* and both whole bodies and feces to provide an adequate spectrum of allergens.⁵⁶

Cockroach has been reported to be an important sensitizer in inner-city pediatric populations.⁵⁷ Ten-year old children from four U.S. inner-city populations who were cockroach sensitive were studied for levels of sIgE to cockroach and eight component allergens.⁵⁷ The 19 who had both rhinitis and asthma compared with the 28 who had neither condition, recognized more and had higher allergen-sIgE levels for cockroach allergens.⁵⁷ Per a 7 was recognized in 52.6% of the children with rhinitis and asthma; otherwise, no allergen was recognized in >50% of the children.⁵⁷ The allergens most closely associated with asthma and rhinitis were Per a 7, Bla g 6, and Bla g 4.⁵⁷ The patterns of sensitization to eight component allergens of cockroach were also studied in 23 adults with cockroach allergy from three U.S. cities.⁵⁸ Different allergens were dominant for different donors. Sensitization to the eight allergens ranged from 30 to 57%, with Bla g 2 and Bla g 11 recognized in >50% of the donors.⁵⁸ In the 15 donors who were most sensitive to cockroach, there were four allergens recognized in >50%, Bla g 2, Bla g 6, Bla g 9, and Bla g 11.⁵⁸

Major allergens have also been identified in fungal extracts.⁵⁹ The presence of sIgE has been found in >50% of the subjects who were sensitized for Alt a 1 from *Alternaria alternata*, Cla h 6, and Cla h 8 from *Cladosporium herbarum*, Asp f 1 from *Aspergillus fumigatus*, and Pen ch 13 and Pen ch 18 from *Penicillium chrysogenum*.

Levels of Major and Minor Allergens in Allergen Extracts

It is common practice to express allergen extract dosing in terms of the amount of a major allergen administered at maintenance. However, it is clear that, in complex extracts that represent environmental exposures, it would be highly desirable to know the amount of all the important component allergens in an extract.

Results of studies show that the profile of component allergens can differ greatly, in part, related to the source material chosen for extraction. Five dog extracts intended for SPTing were analyzed for levels of six component allergens.⁵³ In the commercial SPT extracts, Can f 2 and Can f 6 were very low, whereas Can f 3 (dog albumin) contributed 18 to 98% of the total allergen content.⁵³ Allergen source material from three dog-anatomic sites was also analyzed. Epithelial extract was dominated by Can f 3 (dog albumin), which contributed 99% of the allergen content, whereas 60% of the allergen content of dander extract was also dog albumin, but Can f 1, at 67%, dominated the allergen content of the hair extract.⁵³

The allergen contents of 12 nonstandardized German cockroach extracts and a variety of U.S. cockroach extracts were compared for allergen content and potency of IgE reactivity.⁵⁸ The allergen levels were highly variable. Bla g 1 and Bla g 2 were several times higher in fecal compared with whole-body extracts, whereas the opposite was true for Bla g 5.⁵⁸ Analysis of the 12 German extracts revealed a content of Bla g 1, which averaged 20-fold greater than for Bla g 2, whereas the content of Bla g 5 was very low.⁵⁸ Mold extracts continue to be of very variable composition. An analysis of six commercial mold skin-prick testing solutions in Germany showed the range for Alt a 1 to be 0.18 to 101 $\mu\text{g/mL}$ and for Asp f 1 to be 0.3 to 100 $\mu\text{g/mL}$.⁵⁹ For other molds, the findings were similar.

New Approaches to AIT for Allergy to Cats. There is a prospect for new approaches to AIT for patients with allergy to cats. Studies have been conducted on immunizing cats with the major cat allergen, Fel d 1, plus a virus-like particle from the cucumber mosaic virus, which induce an IgG Fel d 1–neutralizing antibody response.⁶⁰ After immunization, the cats had reduced levels of Fel d 1 in their tears and larger volumes of tears were required for basophil activation. As opposed to this active immunization, cats have been fed the yolks of eggs from chickens that had been immunized with Fel d 1.⁶¹ The yolks from the immunized chickens contain anti-Fel d 1 antibodies that reduced salivary levels of Fel d 1 by 24% over a period of 4 weeks and reduced levels of Fel d 1 deposited on the cats' fur by 47% over 10 weeks. In a clinical trial in patients with cat allergy and who had cats in their homes, background levels of nasal symptoms were not significantly reduced but the patients were more tolerant of intimate contact with their pets (H.J. Wedner, oral communication, October 2019).

Implications. The use of recombinant major allergens and antibodies specific for the major allergens to identify patients' pattern of sensitization and to determine if an allergen extract fits that the patient's pattern could truly make AIT "precision medicine." It is not clear if this can happen without intervention by regulatory authorities, which seems unlikely to occur.

Release of Fel d 1 into the environment can be reduced by active and passive immunization of the cat. The extent to which this will reduce patient's symptoms remains to be determined.

NEW MOLECULES FOR AIT

Finally, there are new molecules for AIT that may be generated from natural allergens (allergoids), which may be the product of recombinant technology or that may be designed to improve the uptake of allergenic material. All of these approaches are sometime in the future because they will require extensive testing to gain FDA approval.

Allergoids

An increasing number of the allergen extracts used in Europe have been modified by exposure to aldehydes to cause structural changes that reduce their reactivity with sIgE while retaining the immunogenicity necessary to induce a favorable T-cell response. An example of the potential advantages of an allergoid is seen in a trial with a birch pollen allergoid in which up-dosing was accomplished in only two visits, each with two injections 30 minutes apart, followed by monthly maintenance injections.⁶² After 1 year of treatment, symptom scores and medication use during the grass pollen season were reduced 34% and 40%, respectively, compared with placebo. It is important that each allergoid be subjected to a controlled trial because methods of manufacturing allergoids differ, and it has been reported that, in some cases, the process of creating the allergoid has reduced the immunogenicity to the same extent as the allergenicity of the extract.⁶³

Recombinant Molecules

Recombinant technology and knowledge of epitopes that react with sIgE and mediate T-cell stimulation allow the manufacture of molecules that avoid reactivity with sIgE and induce desired immunologic responses. A number of approaches have been tried, all with the aim of reducing reactivity with sIgE, thereby, allowing short-course, high-dose treatment; these include the following: a hypoallergenic folding variant of recombinant Bet v 1, which showed efficacy in an EEC after 10 weekly injections⁶⁴; however, a trial that compared preseasonal treatment with the Bet v 1 folding variant and native birch pollen extract found no significant difference after 2 years of treatment between the two forms of AIT for symptom and medication scores, or safety⁶⁵; after four or eight intradermal injections, a mixture of Fel d 1-derived peptides that react with T-cell epitopes but not with sIgE demonstrated persisting benefit when evaluated 2 years later in an EEC⁶⁶; however, a large phase III study showed no difference in nasal symptoms compared

with placebo after 1 year of treatment,⁶⁷ and a phase IIb trial of HDM peptides did not achieve its primary end point, so the program was discontinued.⁶⁸

Three recombinant molecules are undergoing clinical trials in Europe. The first is termed Bet v 1-derived contiguous overlapping peptides.⁶⁹ Three peptides of 49–71 amino acids length were synthesized, which together encompassed the entire bet v 1 molecule with overlapping. When administered in five injections over 4 weeks before the birch pollen season, symptom and medication scores were significantly reduced 26% compared with placebo with the lower (50 μ g) but not with the higher dose; however, a number of the subjects developed late respiratory symptoms, especially after the first two injections.⁶⁹ The subjects were followed up through a second birch pollen season, without further treatment; the symptom reduction compared with placebo was still significant, at 21%.⁷⁰

A second approach was to develop a hypoallergenic vaccine for grass pollen allergy that consists of peptides that were derived from the major IgE-bind sites of the timothy allergens Phl p 1, Phl p 2, Phl p 5, and Phl p 6.⁷¹ The peptides were selected for a lack of IgE binding but also for reduced T-cell proliferation and release of inflammatory cytokines, and for induction of IgG that blocked IgE binding. These peptides were then fused with the hepatitis B-derived PreS domain to serve as the carrier and provide T-cell help. This product was administered as three injections over 2 months, a fall booster, and three injections before the second pollen season in 141 subjects with grass allergy.⁷² Although the primary outcome was not achieved, there was a trend during both seasons for a reduction in nasal and asthma symptoms and the rhinitis quality-of-life questionnaire.

The third preparation is a mixture of peptides, which range from 1 to 10 kDa, generated by enzymatic hydrolysis of purified proteins from *Lolium perenne*.⁷³ In a randomized, double-blind, placebo controlled study, 554 adults who were grass sensitive received two injections of the peptides (total dose, 170 μ g) or placebo at each of four weekly visits.⁷⁴ During the ensuing grass pollen season, symptom and medication scores were reduced 17.9% and Rhinitis Quality of Life Questionnaire (RQLQ) 17.1%, both statistically significant. An immunologic assessment of a subset of these patients revealed blunting of allergen-induced basophil responsiveness, blunting of seasonal increases in levels of grass pollen sIgE, reduction in follicular Th cells, induction of follicular Treg cells and regulatory B cells, induction of IL-35 inducible regulator T-cells (iT_R35) cells, and production of IgG4 antibodies.⁷⁵ In a subsequent study, the subjects were found to tolerate a two injections per visit, seven-visit schedule with a total dose of 490 μ g of these peptides.⁷⁶ This dose was found to have a greater effect on conjunctival allergen challenge than the 170- μ g dose used in reference 75.⁷⁶

Nanoparticles

Encapsulation of allergens or DNA vaccines in nanoparticles of natural or synthetic polymers, liposomes, or virus-like particles can protect the protein-DNA molecule from degradation and prevent reactions with sIgE while providing targeted delivery to the site of the immune response.⁷⁷ Some of these approaches have been applied in studies in the past³⁴ but none are currently being applied in large, human studies. A CpG-motif, G10, without specific allergen, was incorporated into a bacteriophage Q β -derived virus-like particle to stimulate the immune system toward a Th1-mediated response.³⁴ All the parameters reported by the patients with asthma improved over the course of 12 weeks of treatment in the patients treated with Q β G10, despite steroid withdrawal, compared with the deterioration observed in the placebo group. However, the product was subsequently abandoned after a larger trial failed to meet its primary objective.³⁵

Implications. Properly engineered allergoids hold promise to drastically reduce the time spent in up-dosing. It will take a new approach by the FDA to get any of these approved for use in the United States. Thus far, the recombinant molecules have not performed at a level that would encourage the extensive studies necessary for introduction into the United States. It is too early in development to determine whether the nanoparticle will prove effective.

CONCLUSION

In this article, an attempt was made to predict the future of AIT over the next decade. What is the likelihood that these changes will take place? Assessing the efficacy of multiallergen SCIT and SLIT requires trials that are relatively simple to design but that require funding, which thus far has not been available. Funding for research in AIT is largely limited to studies designed to obtain registration for commercial products. The same lack of financial interest applies to dose-response studies for liquid SLIT extracts and use of currently available allergen extracts by alternative routes of administration, such as intralymphatic or epicutaneous. Because it is readily available to anyone, there is also no financial incentive to study the use of vitamin D as an adjuvant. However, it is possible that a company will explore the use of a particular strain of bacteria as a probiotic adjuvant because the data would not apply generically to other strains.

TLR-agonists have been extensively studied as adjuvants in AIT without notable success. It seems unlikely that biologics will play a significant role in routine AIT. The introduction of additional SLIT tablets in the U.S. market is questionable because use of SLIT tablets in

the United States has not been sufficient to encourage the major investment necessary to obtain FDA approval for either the birch or Japanese red cedar tablets, although the latter would probably have a large market in Texas. There is a potential financial incentive for companies to develop microarrays to assess the patterns of sensitization of U.S. patients to allergen extracts that have multiple major sensitizing proteins. If this were to occur, then it might encourage extract companies to analyze and make available information on the major and minor allergen composition of their extracts. Finally, there are modified extracts, allergoids, and recombinant allergens that offer greater safety and, hence, more convenient treatment schedules. For each of these, the manufacturer must determine whether the studies required to obtain FDA approval are worth the risk. Thus, despite the many avenues of possible improvement in AIT, none can be considered inevitable.

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