

# New Types of Immunotherapy in Children

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Injection immunotherapy has been shown to be particularly beneficial in treating allergic rhinitis, mild to moderate asthma, and anaphylaxis caused by bee and wasp venom. It also produces a long-term, antigen-specific, protective immune effect and is the only treatment that offers the possibility of reducing the risk of asthma development in children with allergic rhinitis. Nonetheless, the potentially severe side effects associated with this form of immunotherapy limit its widespread use. Diverse preparations are being developed to increase its safety and improve its efficacy. These include alternative routes of administration, particularly the sublingual route; use of novel adjuvants, such as CpG oligonucleotides and mycobacterial vaccines; and other approaches, such as peptide immunotherapy, recombinant allergens, DNA vaccination, and combined therapy. Some of these immunotherapy forms have been evaluated in children.

## Introduction

Injection immunotherapy is disease modifying and is of proven value in individuals with anaphylaxis caused by bee and wasp venom and in those with allergic rhinitis and bronchial asthma caused by inhaled allergens [1–3]. However, use of whole allergen preparations is associated with the risk of systemic side effects and life-threatening anaphylactic shock during treatment. For these reasons, many approaches to reduce the allergenicity of immunotherapy preparations while maintaining immunogenicity are under development (Table 1) [4]. They include using alternative routes of administration, mainly the sublingual route; the adsorption of allergen extracts to adjuvants to delay the systemic release of allergenic material from

the site of administration and to increase the vaccine's immunogenicity; the combination of allergen extracts with other molecules; the chemical modification of allergen extracts to reduce their allergenic activity; and other approaches, such as peptide immunotherapy, recombinant allergen use, and DNA vaccination [3–6].

This review updates the available evidence about new types of specific immunotherapy with potential use in children in the context of randomized, double-blind, placebo-controlled clinical trials.

## Sublingual Immunotherapy

Sublingual administration of immunotherapy (SLIT) was proposed more than 20 years ago with the main rationale of minimizing the risk of severe adverse events possibly associated with the injection route [7]. After some years and many controlled trials carried out in adults and children [8], SLIT was finally accepted as a viable alternative to the traditional subcutaneous route [9]. SLIT's satisfactory safety profile has been confirmed in clinical trials and postmarketing surveys, even in children younger than 5 years old [10]. Some double-dummy studies failed to detect a significant difference between SLIT and the subcutaneous route [11,12] in terms of clinical efficacy. Moreover, rigorous experimental evidence of the treatment's immunologic effects consistent with the clinical effects also has been provided [13,14••].

Wilson et al. [15] conducted the first Cochrane meta-analysis evaluating SLIT in the treatment of allergic rhinitis. This included 22 trials published up to September 2002 and involved 979 participants. Six trials used SLIT for house dust mite allergy; five for grass pollen; five for *Parietaria* spp; two for olive; and one each for ragweed, cat, tree, and *Cupressus* spp. Five studies enrolled exclusively children. All studies were randomized, double-blind, and placebo-controlled (RCTDBPC) and of parallel group design. Concealment of treatment allocation was considered adequate in all studies, and the use of identical placebo preparations was almost universal. There was significant heterogeneity—most likely due to widely differing scoring systems between studies—for most comparisons. Analysis was performed using standardized mean differences

**Table 1. Immunotherapy approaches currently under investigation**

| References                                                         | Immunotherapy approach                         | Rationale                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|--------------------------------------------------------------------|------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Passalacqua et al. [8], Frew [13], Dahl et al. [14••]              | Sublingual immunotherapy                       | The original rationale for administering immunotherapy sublingually was to achieve prompt and rapid absorption to avoid possible gastrointestinal degradation. Now it is hypothesized that when an allergen is administered sublingually in a high dose, a small proportion of the administered dose is taken up by local dendritic cells through the buccal mucosa. Then, these cells pass to the regional lymph nodes, where they can interact with antigen-specific lymphocytes. Some of the immunologic features of injection immunotherapy are also seen with sublingual administration of immunotherapy. |
| Valenta and Niederberger [5], Valenta [6], Jutel and Cromwell [35] | Recombinant allergens and allergen derivatives | Recombinant allergens can be produced as defined molecules in consistent quality and unlimited amounts according to the corresponding DNA template. Furthermore, they can be modified to reduce their allergenic activity and to foster certain advantageous immunologic properties.                                                                                                                                                                                                                                                                                                                           |
| Larché et al. [4], Larché [27]                                     | Peptide-based immunotherapy                    | Peptides that consist of the amino acid sequences of allergen T-cell epitopes retain the ability to immunomodulate T cells, but these linear molecules, by virtue of their size and relative lack of secondary and tertiary structure, have less ability to cross-link surface IgE and activate effector cells than the corresponding intact allergens.                                                                                                                                                                                                                                                        |
| Walker and Zuany-Amorim [29], Gabler et al. [36]                   | DNA vaccination                                | DNA vaccines incorporate a gene encoding a specific antigen in plasmids that are programmed to induce the synthesis of the antigen under the control of strong eukaryotic promoters. After intramuscular or intradermal injection, the vaccine is taken up by various cells, including antigen-presenting cells. Then, antigen is synthesized by the antigen-presenting cell and seems to induce an allergen-specific T-helper type 1–cell response.                                                                                                                                                           |
| Behrmann [38], Creticos et al. [39]                                | Conjugated molecules                           | To enhance the therapeutic efficacy of allergen vaccines, purified allergens have been conjugated to immunostimulatory DNA moieties. Unmethylated cytosine–guanine dinucleotide DNA sequences, found in certain bacterial species, appear to stimulate the immune system so that the development of a T-helper type 1–type immune response is favored over a T-helper type 2–type response.                                                                                                                                                                                                                    |
| Larché et al. [4], Francis and Durham [41]                         | Novel adjuvants                                | Monophosphoryl lipid A, microencapsulated allergen preparations, and carbohydrate-based particles could provide an alternative to aluminium hydroxide. Killed micobacteria are highly immunostimulatory and evoke strong interferon- $\gamma$ responses in murine models.                                                                                                                                                                                                                                                                                                                                      |
| Kuehr et al. [50]                                                  | Anti-IgE and immunotherapy                     | An RCTDBPC reported that combined therapy reduced symptom scores by 48% in children and adolescents with seasonal allergic rhinitis over 2 pollen seasons.                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| RCTDBPC—randomized, double-blind, and placebo-controlled trial.    |                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |

(SMDs) and the random-effects model. Overall, there was a significant reduction in symptoms (SMD, -0.42; 95% CI, -0.69 to -0.15;  $P = 0.002$ ) and medication requirements (SMD, -0.43; 95% CI, -0.63 to -0.23;  $P = 0.00003$ ) after immunotherapy. An update of this meta-analysis is currently in progress.

In children, a meta-analysis of RCTDBPCs evaluating SLIT in the treatment of allergic rhinitis in patients 18 years of age or younger was carried out with available trials up to February 2006 [16]. Symptom scores and rescue medication use were the outcomes measured. Seventy articles were identified and reviewed. Ten studies fulfilled the selection criteria, all of which were published between 1990 and 2004. Participants with a final clinical assessment ( $n = 484$ ) were analyzed; 245 patients received SLIT, and 239 placebo. A relevant heterogeneity due to widely differing scoring systems was found. Overall, there was a highly significant reduction in symptoms (SMD, -

0.56; 95% CI, -1.01 to -0.10;  $P = 0.02$ ) and medication use (SMD, -0.76; 95% CI, -1.46 to -0.06;  $P = 0.03$ ) after immunotherapy. The subanalysis showed that treatments over 18 months were effective, and SLIT was effective with pollens but not mites.

A meta-analysis of RCTDBPCs evaluating SLIT's efficacy in the treatment of allergic asthma in children was published recently [17]. For this review, electronic databases were searched up to May 31, 2006, and effects on primary outcomes (ie, symptom scores and concomitant use of rescue medication) were calculated with SMD using the random-effects model. Seventy-three articles were identified and reviewed. Nine studies, all published after 1990, fulfilled the selection criteria. A total of 441 patients had a final assessment and were included in the analysis. A total of 232 patients were treated with SLIT, and 209 patients received placebo. Overall, there was a significant reduction in asthma symptoms (SMD, -1.14;

95% CI, -2.10 to -0.18;  $P = 0.02$ ) and medication use (SMD, -1.63; 95% CI, -2.83 to -0.44;  $P = 0.007$ ) after SLIT. Results of the analysis demonstrated a significant heterogeneity due to widely differing scoring systems. The authors concluded that SLIT with standardized extracts reduces symptom scores and rescue medication use in children with allergic asthma compared with placebo.

Since this last review, new studies assessing SLIT efficacy in pediatric patients have been published (Table 2). Valovirta et al. [18••] carried out an RCTDBPC in children with allergic rhinitis and/or asthma. They compared a low-dose ( $n = 32$ ) and high-dose ( $n = 27$ ) schedule of SLIT with tree pollens versus placebo ( $n = 29$ ) for up to 18 months (Table 2). During the birch pollen season, the high-dose group showed a significant reduction of symptom ( $P = 0.01$ ) and medication scores ( $P = 0.04$ ) compared with placebo. The low-dose group showed a significant reduction only in symptom scores ( $P = 0.03$ ). Treatment was generally well tolerated, and the most frequent adverse events were local reactions [18••].

Lue et al. [19] conducted an RCTDBPC in 6- to 12-year-old children with mild to moderate asthma with single sensitization to house dust mites. They were randomized to receive SLIT ( $n = 10$ ) or placebo ( $n = 10$ ) with a standardized extract of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*. The cumulative doses were about 1.7 mg and 3.0 mg, respectively. After 6 months, only patients receiving SLIT presented reductions in daytime ( $P = 0.004$ ) and nighttime ( $P < 0.001$ ) asthma symptoms and medication scores. Serum levels of mite-specific IgG4 increased significantly ( $P < 0.037$ ) in the SLIT group [19].

Röder et al. [20] conducted an RCTDBPC in children 6 to 18 years old who had allergic rhinitis. Jadad score for this trial was 5 of 5. A total of 204 patients were randomized, 24.5% of whom dropped out during the follow-up. Patients receiving SLIT were treated with 9500 biological units (BU) (21- g equivalent Lol p 5) twice weekly for 2 years. Both intention-to-treat and per-protocol analysis of mean daily total symptom score did not reveal a significant difference between SLIT and placebo groups. Also, no differences were found for rescue medication-free days, disease-specific quality of life, or overall evaluation of the treatment effect [20]. A recent multicenter, randomized, double-blind, placebo-controlled, phase 2/3 study (AB0602) assessed these extracts' clinical efficacy and safety in adults with allergic rhinitis after one season. Three different dosages were evaluated: 9500 BU twice weekly, 9500 BU daily, and 19,000 BU daily. Analysis of this study showed that only the highest dosage satisfied the efficacy criterion [21].

Cao et al. [22] published the largest SLIT RCTDBPC including children. Patients were 4 to 18 years old with allergic rhinitis and/or asthma. A total of 208 children were included, 27 (10%) of whom dropped out before study completion. Efficacy analysis evaluated 122 patients receiving

SLIT with *D. farinae* and 129 receiving placebo. After 25 weeks of treatment, there was a significant reduction in nasal symptom scores ( $P < 0.01$ ) and asthma medication scores ( $P < 0.05$ ). Also, allergen-specific IgG4 levels increased significantly in the treated group ( $P < 0.01$ ) [22].

Bernardini et al. [23] recently reported that SLIT during 18 months with natural rubber latex was effective compared with placebo in an RCTDBPC conducted in children with manifestations of asthma, allergic rhinitis, and urticaria associated with latex exposure. Also, Pajno et al. [24] demonstrated that SLIT with mites reduced clinical scores in an 18-month RCTDBPC conducted in children 5 to 16 years old with atopic dermatitis.

SLIT has been demonstrated to be safe in children. In a postmarketing evaluation, Di Rienzo et al. [10] reported only two buccal and five gastrointestinal manifestations in a survey that included 126 children 3 to 5 years old (5.6% of patients and 0.2 of 1000 doses). Fiocchi et al. [25] reported adverse reactions in 11 of 65 children, none of which were severe enough to require discontinuation of immunotherapy. Most adverse effects were reported after SLIT with house dust mites, mainly during the first 30 minutes after administration, and 54% of reactions occurred during the build-up phase [25]. No anaphylaxis or fatalities have been reported in children using SLIT [26].

### Peptide-Based Immunotherapy

One approach to reducing the allergenicity of immunotherapy preparations while maintaining immunogenicity is the use of short synthetic peptides. These peptides, which consist of the amino acid sequences of allergen T-cell epitopes, retain the ability to immunomodulate T cells, but these linear molecules, by virtue of their size and relative lack of secondary and tertiary structure, have less ability to cross-link surface IgE and activate effector cells than the corresponding intact allergens [4,27].

Peptides for treatment are selected from candidate sequences on the basis of their ability to induce proliferation in lymphocyte lines from a panel of patients with a specific allergy [28]. Peptide therapy may disrupt clonal expansion of T cells after re-exposure to allergen and thereby prevent B-cell activation and, subsequently, IgE production. Another advantage is that a large excess of these peptides could be administered, which might further increase the likelihood of T-cell downregulation and induce epitope-specific anergy or tolerance. Moreover, they are easily manufactured and standardized [27,29].

Some allergen sensitization models have been used in preclinical studies, including perennial allergens such as Der p 2 and Fel d 1; seasonal allergens such as Bet v1; and insect venom allergens such as Api m 1, Api m 4, and Dol m 5 [27]. von Garnier et al. [30] conducted a study in which mice were sensitized to Api m 1 and then treated with a mixture of three polypeptides covering the spanning molecule. This intervention resulted in

Table 2. SLIT: randomized, double-blind, placebo-controlled clinical trials in children

| Author                          | Age, y     | Diagnosis | SLIT/placebo* | Duration, mo | Allergen                                                                         | Units*               | Cumulative dose                            | Dropout rate, % | Jadad score (out of 5) |
|---------------------------------|------------|-----------|---------------|--------------|----------------------------------------------------------------------------------|----------------------|--------------------------------------------|-----------------|------------------------|
| Tari et al. [51]                | 5–12       | R, A      | 30/28         | 18           | Mites                                                                            | STU                  | 365 STU                                    | 12              | 4                      |
| Hirsch et al. [52]              | 6–16       | R, A      | 14/15         | 12           | Mites                                                                            | µg                   | 570 µg                                     | 5               | 5                      |
| Vourdas et al. [53]             | 7–17       | R, A, C   | 34/32         | 24           | <i>Olea europaea</i>                                                             | IR                   | Ole e 1 (8.1 mg)                           | 3               | 4                      |
| La Rosa et al. [54]             | 6–14       | R         | 16/17         | 24           | <i>Parietaria judaica</i>                                                        | IR                   | 52.5 mg                                    | 19              | 4                      |
| Novembre et al. [55]            | 4–14       | R, A, C   | 24/20         | 3            | Mixed grasses                                                                    | AU                   | 37,250 AU                                  | 8               | 5                      |
| Pajno et al. [56]               | 8–15       | A         | 12/9          | 24           | Mites                                                                            | BU                   | 250 µg Der p 1;<br>125 µg Der p 2          | 13              | 5                      |
| Ozdemir et al. [57]             | 7–18       | R, A      | 7/7           | 6            | Mites                                                                            | IR                   | 0.56 mg Dpt; 0.98 mg Df                    | 0               | 4                      |
| Ippoliti et al. [58]            | 5–12       | R, A, C   | 18/15         | 6            | <i>Dermatophagoides pteronyssinus</i>                                            | BU                   | 12 mg                                      | 0               | 4                      |
| Wüthrich et al. [59]            | 4–11       | R, C      | 10/12         | 24           | Mixed grasses                                                                    | µg                   | 139.88 µg                                  | 21              | 4                      |
| Bufe et al. [60]                | 9 ± 2.8    | R, A      | 63/63         | 36           | Grass pollen extracts                                                            | AU                   | 9.6 mg                                     | 18              | 5                      |
| Rolnick-Werninghaus et al. [61] | 3–14       | R, A      | 39/38         | 32           | Mixed grasses                                                                    | STU                  | 188 µg                                     | 17              | 5                      |
| Niu et al. [62]                 | 6–12       | R, A      | 49/48         | 6            | Mites                                                                            | IR                   | 1.7 mg Dpt; 3.0 mg Df                      | 12              | 4                      |
| Bernardini et al. [23]          | 4–15       | R, A, U   | 12/8          | 12           | NRL extract                                                                      | µg                   | 15.3 mg NRL                                | 0               | 5                      |
| Valovirta et al. [18••]         | 5–15       | R, A, C   | 27/29         | 19           | <i>Betula verrucosa</i> ,<br><i>Corylus avellana</i> ,<br><i>Alnus glutinosa</i> | Aquagen<br>SQ units† | Low dose: 67.5 µg;<br>high dose: 562.87 µg | 14              | 4                      |
| Lue et al. [19]                 | 6–12       | A         | 10/10         | 6            | Mites                                                                            | IR                   | 1.7 mg Dpt; 3.0 mg Df                      | 0               | 4                      |
| Pajno et al. [24]               | 5–16       | AD        | 26/22         | 18           | Mites                                                                            | µg                   | 240 µg Der p 1; 195 µg Der f 1             | 15              | 5                      |
| Röder et al. [20]               | 12.9 ± 2.6 | R         | 82/72         | 24           | Mixed grasses                                                                    | BU                   | 4.5 mg Lol p 5                             | 25              | 5                      |
| Cao et al. [22]                 | 4–18       | R, A      | 122/129       | 6            | <i>Dermatophagoides farinae</i>                                                  | µg                   | 6.3 mg Df                                  | 10              | 4                      |

\*Patients having a final assessment.

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A—asthma; AD—atopic dermatitis; AU—allergic units; BU—biological units; C—conjunctivitis; IR—index of reaction; NRL—natural rubber latex; R—rhinitis; SLIT—sublingual immunotherapy; STU—specific treatment units; U—urticaria.

mice anaphylaxis protection. Also, a significant reduction in specific IgE and an increase in allergen-specific IgG2a were observed [30].

Initial clinical trials reported frequent adverse events, mostly related to high doses of peptides. More recently, low doses of peptides have been shown to modify clinical outcomes and biomarkers without inducing adverse events. Current results indicate that peptide-based therapy modulates the immune response by reducing T-helper type 2 (Th2) responses to allergen and increasing interleukin-10 production and the activity of allergen-specific T regulatory cells. Peptide immunotherapy in clinical studies is restricted to peptides from three model allergens: Fel d1 from cats, Api m1 (PLA2) from bee venom, and the ragweed allergen Amb a 1 [27].

In this regard, Alexander et al. [31] conducted an RCTDBPC in 16 cat-allergic asthmatic patients who were randomly assigned to receive Fel d 1 peptides or placebo. In the active group (but not the placebo), there were significant decreases in the late asthmatic reaction to whole cat dander ( $P = 0.03$ ) after 4 months but no between-group difference [31]. Tarzi et al. [32] carried out an open trial in which 12 volunteers with allergy to bee venom received nine intradermal injections of HLA-DR-based PLA2 peptides, with six untreated participants serving as controls. Those receiving peptides showed a decrease in the magnitude of the late-phase cutaneous reaction to bee venom compared with controls ( $P = 0.03$ ). The proliferation of venom-stimulated peripheral blood mononuclear cells decreased in treated patients compared with controls ( $P = 0.01$ ) [32]. Studies to define the optimal dose, dose interval, and route of administration are in progress [27].

## Recombinant Allergens

Therapeutic vaccines are produced from extracts of natural source materials such as grass pollen. Extracts also contain nonallergenic proteins that are not thought to be relevant to treatment, and some have been shown to prime Th2 responses [6,33]. In other cases, important allergens are present in small amounts or lacking, and their biologic potency is subject to huge variability [5,34].

Recombinant allergens can be produced as defined molecules in consistent quality and unlimited amounts according to the corresponding DNA template. Furthermore, they can be modified to reduce their allergenic activity [5]. The use of recombinant DNA technology makes it possible for allergens to be produced to high standards, resulting in preparations with improved quality in terms of purity, consistency, composition, and dosage and the possibility of creating allergen derivatives with reduced IgE reactivity. Vaccines also can be formulated to include only the most relevant allergens in defined concentrations and can be matched to allergen sensitizations of individual patients. Many allergens have

now been cloned for research purposes, but only a few have been developed to the stage at which they can be used in clinical studies [6,33,35].

Jutel and Cromwell [35] have described the development process of recombinant allergen-based vaccines. The first important step is the selection of the predominant allergen sources. Then, messenger RNA is isolated and used as a template to synthesize complementary DNA (cDNA). The next step is to insert the cDNA into a suitable vector (viral and plasmid DNA), which enables it to be expressed and produced in a suitable host organism. After a fermentation process and induction of gene expression, the heterologous recombinant protein is recovered from the cell culture and purified using appropriate chromatographic methods. A master cell bank incorporating a DNA construct including the expression vector together with the cDNA encoding the protein is created. Each production run starts with an aliquot of cells derived from the one cell bank, thus ensuring that the identical protein is always produced [35]. Further steps are quality control, purity determination, allergen concentration confirmation, potency assessment, and natural allergen equivalency. Levels of possible contaminants, including endotoxin and host-cell proteins, are also measured [5,33,35].

According to Valenta and Niederberger [5,6], important new allergens are often discovered by molecular cloning techniques and not by protein chemical methods. In these cases, it is important to obtain as complete a panel as possible of recombinant allergens via IgE-based screening approaches and to evaluate the individual components' relevance. Because IgE serology is not always a good predictor of the allergenic activity, skin test studies performed in large patient populations may be used to identify the most clinically relevant allergen molecules to be included in a therapeutic vaccine. Information regarding the continuously growing number of recombinant allergen molecules can be extracted from databases [5,6].

Jutel et al. [33] carried out an RCTDBPC to evaluate the efficacy of a mixture of five recombinant grass pollen allergens in adults allergic to grass pollen. Allergens were administered subcutaneously for up to 20 months. Evaluation of symptoms scores for per-protocol set showed a 36.5% lower median average symptom score for active treatment compared with placebo (3.38 vs 5.32;  $P = 0.015$ ). Medication requirements were also reduced, as reflected in a 36.5% lower medication score. The rhinitis quality-of-life questionnaire revealed clinically relevant significant improvements favoring active treatment. All treated patients developed strong allergen-specific IgG1 and IgG4 antibody responses. Some patients were not sensitized to Phl p 5 but nevertheless developed strong IgG antibody responses to that allergen. A total of 122 adverse events related to treatment were recorded, 78 in patients receiving immunotherapy

(10.7% of 731 injections) and 44 in the placebo group (5.9% of 748 injections) [33].

### DNA Vaccination

Vaccines with allergen-encoding DNA are being investigated as a potential strategy for SLIT. DNA vaccines incorporate a gene encoding a specific antigen in plasmids that are programmed to induce synthesis of the antigen under the control of strong eukaryotic promoters. After intramuscular or intradermal injection, the vaccine is taken up by various cells, including antigen-presenting cells. Antigen is then synthesized by the antigen-presenting cell and seems to induce an allergen-specific Th1-cell response [29].

Allergen-encoding DNA vaccines administered prophylactically in murine models prevented allergic sensitization [4]. Gabler et al. [36] conducted a study in which a Sindbis virus replicon-based DNA vaccine encoding the major timothy grass pollen allergen Phl p 5 was evaluated for its antiallergic potential compared with a conventional DNA vaccine in a BALB/c murine model of allergy. Mice were intradermally prevaccinated with plasmid DNA, followed by sensitization and intranasal allergen provocation with recombinant Phl p 5. Both vaccines induced Th1-biased immune responses, resulting in suppression of functional IgE, reduction of eosinophilia in bronchoalveolar lavage fluid, and alleviation of lung pathology. However, immunization with the replicon DNA vaccine elicited these effects at a 100-fold lower dose compared with the conventional DNA vaccine. Replicase-based DNA vaccines stimulate Th1-biased immune responses at ultralow doses and induce self-removal of transfected cells through apoptosis. Both aspects are important requirements for efficient and safe DNA-based immunotherapy for type I allergies [36].

Kim et al. [37] evaluated the effects of vaccination with mixed naked DNA plasmids encoding Der p 1, Der p 2, Der p 3, Der f 1, Der f 2, and Der f 3, the major house dust mite allergens on the allergic inflammation, to the whole house dust mites crude extract. A total of 300  $\mu$ g of these gene mixtures was injected into muscle of BALB/c mice. Control mice were injected with the pcDNA 3.1 blank vector. After 3 weeks, the mice were actively sensitized and inhaled the whole house dust mite extract intranasally. The vaccinated mice showed a significantly decreased synthesis of total and house dust mite-specific IgE compared with controls. Analysis of the cytokine profile of lymphocytes after challenge with house dust mite crude extract revealed that mRNA expression of interferon- $\gamma$  was higher in the vaccinated mice than in the controls. Reduced infiltration of inflammatory cells and the prominent infiltration of CD8<sup>+</sup> T cells were observed in lung tissue histology from the vaccinated mice [37].

Plasmid DNA vaccine is produced endogenously and presented to the immune system at a very low concen-

tration over a prolonged period. The slow production of allergen reduces the risk of a severe hyperimmune response, as can happen with conventional allergenic extracts. Consequently, life-threatening systemic reactions appear unlikely with plasmid DNA vaccines, which would further increase the safety and utility of SLIT. Vaccination with allergen-encoding DNA provides a promising approach for treating allergic diseases, but further evaluations are required [38].

### Conjugated Molecules, Novel Adjuvants, and Immunotherapy With Mycobacteria

#### Conjugated molecules

To enhance the therapeutic efficacy of allergen vaccines, purified allergens have been conjugated to immunostimulatory DNA moieties. Targeting Toll-like receptors (TLRs) with TLR ligands linked to allergens has been proposed as a potentially effective strategy. Unmethylated cytosine-guanine dinucleotide DNA sequences, found in certain bacterial species, possess immunostimulatory capacities. DNA conjugates appear to stimulate the immune system so that the development of a Th1-type immune response is favored over a Th2-type response. Preliminary studies have demonstrated that immunostimulatory DNA-conjugate allergen products have the potential to be several folds less allergenic than conventional allergenic extracts [38].

Creticos et al. [39] conducted an RCTDBPC of a vaccine consisting of Amb a 1, a ragweed pollen antigen, conjugated to a phosphorothioate oligodeoxyribonucleotide immunostimulatory sequence of DNA (AIC) in 25 adults who were allergic to ragweed. Patients received six weekly injections of the AIC or placebo vaccine before the first ragweed season and were followed up during two ragweed seasons. During the first ragweed season, the AIC group had better peak season rhinitis scores ( $P = 0.006$ ), peak season daily nasal symptom diary scores ( $P = 0.02$ ), and midseason overall quality-of-life scores ( $P = 0.05$ ) than the placebo group. AIC did not alter the primary end point, the vascular permeability response (measured by the albumin level in nasal lavage fluid) to nasal provocation. AIC induced a transient increase in Amb a 1-specific IgG antibody but suppressed the seasonal increase in Amb a 1-specific IgE antibody. A reduction in the number of interleukin-4-positive basophils in AIC-treated patients correlated with lower rhinitis visual analogue scores ( $r = 0.49$ ;  $P = 0.03$ ). Clinical benefits of AIC were again observed in the subsequent ragweed season, with improvements over placebo in peak season rhinitis visual analogue scores ( $P = 0.02$ ) and peak season daily nasal symptom diary scores ( $P = 0.02$ ). The seasonal-specific IgE antibody response was again suppressed, with no significant change in IgE antibody titer during the ragweed season ( $P = 0.19$ ) [39].

Nayak et al. [40] recently evaluated the safety and immunogenicity of a novel ragweed vaccine in children. In this preliminary report, individuals 6 to 17 years old with documented ragweed allergy received up to 30 µg of an amb a 1-1018 immunostimulatory oligodeoxyribonucleotide conjugate vaccine ( $n = 6$ ) or placebo ( $n = 6$ ) by subcutaneous injection. All patients in each cohort received a complete set of six weekly immunizations. Most adverse events reported were mild and consisted primarily of injection site reactions. Two transient, severe adverse events occurred in the AIC group: injection site pruritis and flank pain. No serious adverse events occurred and no significant differences between groups were observed for rhinitis symptoms, visual analogue scale scores, change from baseline skin test reactivity, or relief medication use. The placebo group had greater increases in IgE antiragweed antibodies; the AIC group had greater increases in IgG antiragweed antibodies [40]. Vaccines of allergens conjugated to immunostimulatory DNA moieties could ameliorate the safety and efficacy of future SLIT regimes, but additional evaluations are required.

### Adjuvants

Aluminium hydroxide remains the most common injection immunotherapy in humans. This has proven to be efficacious and has a good safety profile. Although recent studies have indicated that it downregulates human Th2-cell responses to allergens in vitro, there are several disadvantages to its use, including increased sensitivity to aluminium and local granuloma formation at injection sites [41].

Monophosphoryl lipid A, a bacterial cell wall component that binds TLR4, microencapsulated allergen preparations, and carbohydrate-based particles, could provide an alternative to aluminium hydroxide [4,41].

### Immunotherapy with mycobacteria

Heat-killed *Mycobacterium vaccae* has prevented allergic and asthmatic manifestations and ameliorated airway histopathology in asthma murine models [42]. Killed mycobacteria are highly immunostimulatory and evoke strong interferon- $\gamma$  responses in murine models [41]. *M. vaccae* previously has been shown to successfully treat children with moderate to severe atopic dermatitis [43]. However, recent data are conflicting. In one recent RCTDBPC including 166 children, *M. vaccae* administration in patients with moderate to severe atopic dermatitis was no more effective than placebo in ameliorating atopic dermatitis severity [44]. In another RCTDBPC carried out in 24 adults with asthma, patients treated with *M. vaccae* showed a reduction of the forced expiratory volume in 1 second (FEV<sub>1</sub>) changes during the late asthmatic response, but this was not significantly different from placebo [45].

*Mycobacterium bovis* bacille Calmette-Guérin (BCG) vaccination has been shown to inhibit allergic airway inflammation and reduce airway hyperresponsiveness and eosinophilia in animal models [46]. Results are controversial in humans. An RCTDB conducted in children reported that simultaneous BCG vaccination at the initiation of a SLIT schedule provided no additional benefit compared with those patients receiving only SLIT [47]. In an adult RCTDBPC, 31 Mantoux-negative adults with stable, moderately severe asthma who were skin prick test-positive to house dust mite were randomized to receive one injection (0.1 mL) per week for 4 weeks of heat-inactivated BCG or normal saline. No significant differences in markers of asthma severity, symptom and medication scores, FEV<sub>1</sub>, blood eosinophil counts, or IgE levels were observed after 3 months [48].

### Anti-IgE and Immunotherapy

The monoclonal anti-IgE antibody omalizumab lowers levels of serum-free IgE in humans and reduces IgE-mediated symptoms, regardless of the allergen specificity of the IgE involved. Efficacy of anti-IgE versus placebo in reducing the symptom severity of seasonal allergic rhinitis and allergic asthma and in improving rhinoconjunctivitis-specific quality of life has been reported [49,50].

Kuehr et al. [50] conducted an RCTDBPC to assess the efficacy and safety of subcutaneously administered anti-IgE (omalizumab) or placebo in children and adolescents with seasonal allergic rhinitis in a birch pollen season and a grass pollen season. There were four treatment arms. Each patient was started on SLIT-birch or SLIT-grass, and anti-IgE or placebo was started before and maintained during the anticipated pollen seasons (a total of 24 weeks). A total of 221 patients 6 to 17 years old were analyzed for efficacy. Combined therapy reduced symptom load over the two pollen seasons by 48% ( $P < 0.001$ ) over SLIT alone [50].

### Conclusions

Evidence presented in RCTDBPCs and meta-analyses suggests that SLIT is an effective method of immunotherapy administration for treating allergic rhinitis and asthma in children. Further evaluations of SLIT's efficacy in atopic dermatitis and latex allergy are required. Promising results have come from studies using immunostimulatory oligodeoxyribonucleotide conjugate vaccines and immunotherapy plus anti-IgE antibodies, but additional clinical trials of these approaches have to be conducted. Many immunotherapy approaches are currently at pre-clinical or early clinical stages; consequently, further research is required before any therapeutic proposal in allergic children can be made.

## Disclosures

No potential conflicts of interest relevant to this article were reported.

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