

For reprint orders, please contact reprints@expert-reviews.com



A comparison of immunotherapy delivery methods for allergen immunotherapy

Expert Rev. Clin. Immunol. 9(5), 465–475 (2013)

Ludger Klimek* and Oliver Pfaar

Allergy Center, An den Quellen 10,
D-65183 Wiesbaden, Germany

*Author for correspondence:

Tel.: +49 611 308 6080

ludger.klimek@allergiezentrum.org

Background: Allergic diseases are among the most common diseases in humans. Besides allergen avoidance, allergen-specific immunotherapy is the only causative treatment option. During recent years, many innovations of this therapy have emerged. **Methods:** Selective literature research in Medline and PubMed, under the inclusion of national and international guidelines and Cochrane meta analyses. **Results:** In several meta-analyses, the clinical efficacy of subcutaneous immunotherapy (SCIT) has been largely demonstrated. Recently, major research activities in mucosal immunotherapies focused on the sublingual application route. There are well-documented clinical data on the efficacy and safety of this form of immunotherapy. New application routes as well as new immune-modifying agents such as virus-like particles or CpG-motifs have also been investigated. **Conclusion:** SIT is accepted to be the only causative treatment option for allergies. New application routes and new immune-modifying agents will allow for different delivery methods in the future.

KEYWORDS: adjuvants • allergen-specific immunotherapy • CpG-motifs • epicutaneous immunotherapy • intralymphatic immunotherapy • subcutaneous immunotherapy • sublingual immunotherapy • virus-like-particles



Medscape: Continuing Medical Education Online

This activity has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education through the joint sponsorship of Medscape, LLC and Expert Reviews Ltd. Medscape, LLC is accredited by the ACCME to provide continuing medical education for physicians.

Medscape, LLC designates this Journal-based CME activity for a maximum of 1 **AMA PRA Category 1 Credit(s)**[™]. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

All other clinicians completing this activity will be issued a certificate of participation. To participate in this journal CME activity: (1) review the learning objectives and author disclosures; (2) study the education content; (3) take the post-test with a 70% minimum passing score and complete the evaluation at www.medscape.org/journal/clinicalimmunol; (4) view/print certificate.

Release date: 1 May 2013; **Expiration date:** 1 May 2014

Learning objectives

Upon completion of this activity, participants will be able to:

- Assess the epidemiology of allergic disease
- Analyze the practice of SCIT
- Distinguish the primary and secondary sites of application of immunotherapy
- Evaluate the effects of adjuvants in immunotherapy

Financial & competing interests disclosure**EDITOR**

Eliza Manzotti

Publisher, Future Science Group, London, UK

Disclosure: Eliza Manzotti has disclosed no relevant financial relationships.

CME AUTHOR

Charles P Vega, MD

Associate Professor and Residency Director, Department of Family Medicine, University of California, Irvine, CA, USA

Disclosure: Charles P Vega, MD, has disclosed no relevant financial relationships.

AUTHORS AND CREDENTIALS

Ludger Klimek

Allergy Center, An den Quellen 10, D-65183 Wiesbaden, Germany

Disclosure: Ludger Klimek has received honoraria from ALK-Abello, Allergopharma, Bionorica, Boehringer Ingelheim, GlaxoSmithKline, Lofarma, Novartis, MEDA Pharma, MSD, Phadia/ThermoFisher and Optima, and has acted as a clinical study investigator for ALK-Abello, Allergopharma, Artu-Biologicals, Bencard, Bionorica, Biomay, Cytos, HAL Allergy, Hartington, GlaxoSmithKline, Leti, Lofarma, Novartis and Roxall.

Oliver Pfaar

Allergy Center, An den Quellen 10, D-65183 Wiesbaden, Germany

Disclosure: Oliver Pfaar has received research grants from and/or has served as an advisor and on the speakers' bureaus for ALK-Abello, Allergopharma, Stallergenes, HAL, Artu Biologicals, Allergy-Therapeutics/Bencard, Hartington, Lofarma, Novartis/Leti, GlaxoSmithKline, Essex-Pharma, Cytos, Curalogic, Roxall, Biomay, ThermoFisher and MEDA Pharma, has received travel grants from HAL Allergy and Allergopharma, and is a consultant for HAL Allergy.

The increase in allergic diseases to ubiquitous aeroallergens in recent decades has been well documented [1]. Worldwide, the prevalence of self-reported rhinitis symptoms in adolescents 13–14 years of age is reported to be in the range of up to 66% [2]. In Germany, 24% of respondents suffered from seasonal allergic rhinitis to pollen [2]. This fits to the number of approximately 25% of the population that is said to suffer from allergic rhinoconjunctivitis (AR) in Germany, and of approximately 5% that suffer from allergic asthma [3].

Today, AR is divided into intermittent AR (<4 weeks/year) and persistent AR (>4 weeks/year). Moderate-to-severe symptoms are present if nasal or eye symptoms in untreated patients affect their daily activities and/or affect their night's sleep. An inhalation allergy must always be considered if the symptoms occur preferentially in certain seasons or in certain situations. The allergy career often starts in childhood with atopic dermatitis and food allergies. Airway symptoms such as allergic asthma and allergic rhinoconjunctivitis follow later on.

Allergies are often trivialized and regarded more as inconveniences than real diseases [4]. According to a recent survey [101], about 58% of allergy sufferers treat their allergy with nonprescription drugs or allergen avoidance.

Allergy treatment is aimed at treatment based on symptoms of the disease and preventing disease progression. Allergen-specific immunotherapy (also called specific immunotherapy, hypo-sensitization therapy, desensitization, allergen vaccination or allergen immunotherapy), the administration of gradually increasing amounts of an allergen, either subcutaneously or via the sublingual or oral route, is effective. According to the abovementioned survey, 28% of the respondents received allergen-specific immunotherapy (SIT), and 70% of them were satisfied with the results of SIT [101]. SIT is the only treatment that is shown to have a disease-modifying effect and can also be successfully performed in childhood [5,6].

The socioeconomic costs for respiratory allergies in Europe are estimated to be between €36.7 and €385.1 billion per year [7]. More advanced diseases are more difficult to treat and associated with higher treatment costs. Thus, the total annual cost for a patient with mild asthma is approximately €1670, and for a patient with severe asthma approximately €6008 [8]. In two reports SCIT has been analyzed to be cost effective in the seventh year after initiation of therapy [7,8].

Methods

For the present review, a literature search has been performed including the Cochrane Database [9–11], the German guideline for SIT [12], as well as the European position papers of EAACI [6,13,14] and GA2LEN/EAACI [15], the recommendations of the WHO [16,17], the recommendations of the British Society for Allergy and Clinical Immunology [18], and the CHMP guidelines [102,103]. In addition, the results of a selective search in PubMed and Medline (main keywords: allergen-specific immunotherapy, application, mucosal immunotherapy) have been included. Large double-blind, placebo-controlled (DBPC) studies during recent years have in particular been selected.

Major features of allergen immunotherapy***Treatment of allergic diseases of the respiratory tract***

Allergen avoidance is the first-line treatment of allergic diseases in general, but is often not sufficient for mite and pollen allergies. For the symptomatic treatment of allergic rhinoconjunctivitis, antihistamines, topical and systemic corticosteroids, and leukotriene receptor antagonists are available. However, these drugs are purely symptomatic but no curative treatment is available. The effectiveness of SIT in AR has been shown in many clinical studies. Meta-analyses summarize the results of different studies (different

allergen preparations, doses and application routes) [9,10]. In the meta-analysis on the efficacy of subcutaneous immunotherapy (SCIT) in allergic asthma, the standardized mean difference (SMD) for symptom score was shown to be -0.59 (-0.83 to -0.35) and for medication score to be -0.53 (-0.80 to -0.27) [11].

Studies on the efficacy of SIT and symptomatic therapies differ in their design. The efficacy of SCIT has been shown to be superior to that of symptomatic drug treatment, even after the first year of treatment [19]. A dose–response curve could be defined for SIT [13].

Monosensitized patients with early-stage disease are thought to be particularly successful candidates for SIT. From a pediatric perspective, an early start of SIT is recommended. An effective therapy is also possible in polysensitized patients [20]. The longest follow-up is published for SCIT with a grass pollen allergoid; here the effect was still detectable 12 years after the end of therapy [21].

Implementation of SIT

Recommendations for the up-titration of SIT and the completion of treatment have been published in national and international guidelines. They are based on clinical studies and published meta-analyses [9–18]. SIT is started with the introduction of treatment during which the dose is increased to the maintenance dose. For native allergens up to 20 weeks may be required for up-titration, while for allergoids shorter periods – or even a 1-day schedule may be sufficient.

Depending on the preparation, treatment may be necessary throughout the year or just preseasonally, and individual allergoids are approved for both treatment regimens. An adequate comparison of the efficacy of different treatment regimens has not been published.

Nonadverse events

The side effects of SCIT include particular allergic reactions (generalized skin reactions and systemic allergic reactions) up to severe anaphylaxis. Official statistics on severe reactions have not been reported, but it is known that in recent years, cases of fatal anaphylaxis have occurred [12]. To mitigate frequent local reactions in the initial treatment phase, a pretreatment with antihistamines is helpful [12]. Compared with SCIT, in SLIT mild side effects in the oral mucosa and pharynx are often seen, while serious side effects are rare. To the authors knowledge, fatal side effects of SLIT have not been reported thus far.

How does allergen-specific immunotherapy work?

To our current knowledge, many immunological mechanisms are complementarily involved in the immune response to SIT such as ‘blocking’ IgG-bodies, antigen presentation and effector cells. Depending on the dose of allergen, the immune system develops immunological tolerance mechanisms by confrontation with biologically high doses of allergen. This leads to an altered balance between Th cells and regulatory T cells [22]. Both naturally occurring thymus-derived FOXP3⁺CD4⁺CD25⁺ regulatory T cells and inducible type 1 regulatory T cells suppress the development of allergic diseases via several mechanisms including suppression of dendritic cells, Th cells, mast cells, eosinophils and basophils; suppression of inflammatory cell migration to tissues; and decrease of

the ratio between allergen-specific IgE and IgG4 antibodies [22,23]. These effects are mainly mediated by the suppressive cytokines IL-10 and TGF- β . However, the exact mechanism of action of SIT has not yet been conclusively elucidated [22,23].

Dose dependency of the immunological effect: definition of an optimal therapeutic dose

In clinical trials a dose–response relationship for SIT has been seen [13]. With a mite extract containing 7 μ g Der p 1 per injection during the maintenance phase, a clinical effect was achieved, which does not increase significantly when 21 μ g Der p 1 were used, but there were more adverse events with the higher dose. A dose of 0.7 μ g Der p 1 did not show effects beyond the placebo effect level [24].

The efficacy of approximately 8 μ g group-5-grass pollen allergen in a tablet was in the range of placebo, the maximum effect was demonstrated with the threefold amount (25 μ g daily), and further increases in the dose had no additional benefit [25]. These studies suggest that it is possible for SCIT and SLIT to define optimal allergen doses.

Requirements for allergen extracts for SIT

Currently in Europe, the classification, composition and quality requirements of allergen extracts are regulated by technical guidelines. The most important change of recent years concerns the definition of homologous groups, based on the structural similarity of allergens, and less on the botanical relationship of allergen sources [102]. Another guideline of the EMA provides recommendations for conducting clinical trials with allergen products that will be developed in the future with gradual dose–escalation and DBPC efficacy trials [103].

Quantification of allergens for immunotherapy

Standardization and characterization of allergen extracts was previously manufacturer-specific only. The determination of the strength of the allergen extract is based on protein determination, electrochemical methods (e.g., immunoelectrophoresis), the size of the wheal and flare reaction in skin tests (prick and intracutaneous tests), and *in vitro* inhibition tests (RAST inhibition). Today, the development of validated methods for the quantification of allergens is of central importance. Allergenic extracts include proteins, glycoproteins, lectins, DNA, pigments, and so on. The immune system interacts with only a few allergens, which are referred to as major allergens if more than 50% of the sensitized patients react to this allergenic protein. As of today, for commercial allergen extracts the declaration of major allergen concentrations is required. A source of allergens has only one major allergen (e.g., Bet v 1 in a birch pollen extract), which is unproblematic. However, more difficult is the characterization of extracts in which different proteins act as major allergens, such as in grass pollen extracts.

The quantification of individual proteins is complicated by the fact that allergens in nature occur in different isoforms with varying biological activity. This may affect the quantification of the proteins by immunological detection methods.

A comparison of the major allergen concentrations in extracts requires validated analytical methods and reference allergens. In a

continuation of the European CREATE project [26], a laboratory ring trial (BSP90) with support from the European Directorate for Quality in Medicine is currently proofing biological reagents and standardization methods for the analysis of allergens in extracts. After publication in the European Pharmacopoeia, these will become mandatory within the EU. The manufacturers declared that allergen concentrations are not sufficient for the evaluation of allergenic extract, thus clinical trials to demonstrate the efficacy of each allergen extract are essential.

Selection of allergen products

In principle, (native) unmodified and modified allergen extracts (allergoids) can be distinguished. Allergoids are produced by treating allergens with formaldehyde or glutaraldehyde, and these modified proteins are thought to have a reduced IgE-binding capacity. In most commercial extracts, aluminum serves as an adsorbent for delaying the release of the allergen. Some products contain additional adjuvants to enhance the immunological effect. Clinical trials of the efficacy of allergoids have been conducted for extracts of grass pollen [27–29], tree pollen [30] and mites [31,32].

Based on the study data, the highest level of evidence for the effectiveness of SCIT and SLIT is given with grass pollen extracts. Grass pollen tablets (SLIT) have been officially approved based on clinical trials for adults and children.

Sublingual immunotherapy

Long-term results, which document a persistent post-treatment effect of SLIT for adults, have been published [33,34]; efficacy on allergic asthma, asthma preventive effects and preventive effects in terms of avoiding new sensitizations have not been sufficiently investigated; however, the results of large prospective studies will be available in the next few years [35]. The database for SLIT with other allergens is mainly considered as insufficient [12]. Some extracts from other sources of allergens have not been thoroughly investigated in clinical trials but have attained the status of an approved product. The approval status of allergen products is available on the Internet [104]. SLIT is conducted in a perennial treatment scheme or pre/co-seasonally. In an average patient, only the first intake needs doctoral supervision. Care must be taken on an intact oral mucosa, since increased side effects may occur with oral mucosa lesions. Therefore, after tooth extractions or gum treatments, therapy may be paused. Treatment should not be carried out immediately after teeth brushing. Studies on the onset of action of SLIT with a grass pollen tablet showed a significant effect of the therapy 30 days after the start of treatment [36].

Experimental routes for delivery of immunotherapy

As shown above, the subcutaneous and sublingual applications of allergen extracts are well established. As experimental alternatives, intralymphatic immunotherapy (ILIT) and epicutaneous immunotherapy as delivery routes for allergen application have been described in recent years.

Senti *et al.* were able to demonstrate the same clinical efficacy with only three intralymphatic injections within 8 weeks using a 100-times reduced cumulative allergen dose if compared with

a 3-year conventional SCIT. The intralymphatic injections were judged by patients to be less painful than venous blood samples. The rate of side effects was significantly lower than in the conventional SCIT and compliance was higher due to the low number of injections [37,38].

In a similar approach, this group further improved intralymphatic allergen administration by targeting allergen to the MHC class II pathway using a recombinant major cat dander allergen, Fel d 1, that had been fused to a translocation sequence (TAT) and to part of the human invariant chain, generating a modular antigen transporter (MAT) vaccine (MAT-Fel d 1) [39].

In a randomized, double-blind clinical study, ILIT with MAT-Fel d 1 in alum was compared with ILIT with placebo (saline in alum) in allergic patients. The authors demonstrated that ILIT with MAT-Fel d 1 elicited no adverse events. After three placebo injections within 2 months, the tolerated allergen dose in nasal allergen challenges increased less than threefold, whereas three intralymphatic injections with MAT-Fel d 1 increased the tolerated dose in nasal challenge 74-fold ($p < 0.001$ vs placebo). ILIT with MAT-Fel d 1 stimulated regulatory T-cell responses ($p = 0.026$ vs placebo) and increased cat dander-specific IgG(4) levels by 5.66-fold ($p = 0.003$). The IgG(4) response positively correlated with IL-10 production ($p < 0.001$). Thus, the authors concluded that in this first-in-human clinical study, ILIT with MAT-Fel d 1 was safe and induced nasal allergen tolerance after three injections.

Kündig discussed the advantages of ILIT and counted the low number of only three injections and the low allergen dose required in comparison to subcutaneous and even more to sublingual administration as major advantages [40].

Senti *et al.* claimed that the allergen dose in ILIT, if compared with SCIT, could be reduced 10–100-fold [41]. They stated that intralymphatic injections proved easy to administer, practically painless and safe, and that in mice and men, intralymphatic immunotherapy injecting allergens into a subcutaneous lymph node markedly enhanced the protective immune response, so that both the dose and number of allergen injections could be reduced, making SIT safer and faster, which enhances patient convenience and compliance.

The same group, Senti *et al.*, for the first time used epicutaneous allergen administration using a patch as an alternative method of allergen delivery in immunotherapy [38]. This transcutaneous route should improve patient compliance and safety. In a monocentric, placebo-controlled, double-blind trial, the authors included 37 adult patients with positive skin prick and nasal challenge tests to grass allergen. These patients were then randomized to a group with allergen patches ($n = 21$) or the second group receiving placebo patches ($n = 16$). Patients were treated before and during the 2006 pollen season, and follow-up visits took place before ($n = 26$) and after the 2007 pollen season ($n = 30$). The results in the nasal challenge tests were the primary outcome measures.

In verum-treated patients, the scores in nasal challenge tests in the first ($p < 0.001$) and second year ($p = 0.003$) significantly decreased. In placebo-treated patients, the scores decreased after 1 year of treatment in 2006 ($p = 0.03$), but the effect diminished in the second year ($p = 0.53$). The improvement of nasal-challenge

test scores was not significantly better in the verum versus placebo group. However, overall treatment success was rated significantly higher by the allergen-treated group than by the placebo group (2006: $p = 0.02$; 2007: $p = 0.005$). Eczema after allergen patch application was seen as a local adverse event (AE); however, it was noted as proof of the local stimulation of specific T-cell responses by the treatment. No severe adverse events were observed.

Another monocentric, randomized DBPC trial was performed on 132 patients with grass pollen-induced rhinoconjunctivitis by the same group using a dose–response model [42]. Patients received either placebo or three different doses of allergen. A total of six weekly patches were applied before and during the 2008 pollen season. After 4–5 months ($n = 110$) and during the pollen season of the treatment-free follow-up year in 2009 ($n = 93$), efficacy measurements were performed. Patient-reported changes in hay fever symptoms were used as primary outcome measures. A visual analog scale was used for this measurement. In addition, the use of rescue medication, changes in conjunctival and skin reactivity, as well as safety parameters were detected as secondary outcome measures.

Symptoms of allergic rhinoconjunctivitis were reduced by more than 30% during the 2008 grass pollen season and by 24% during the 2009 grass pollen season in the high-dose group compared with that in the placebo group. The alleviation of symptoms in the follow-up year was dependent on the treatment dose. Drug-related AEs occurred in the higher allergen doses, predominantly manifested by pruritus, erythema, wheal or eczema. Eleven systemic grade 1–2 AEs of required treatment and led to study exclusion. The dropout rate due to AEs was 8.3%. No serious drug-related AEs were recorded. Thus, the authors concluded that epicutaneous immunotherapy is safe and efficacious in a dose-dependent manner after only six patches.

The authors are of the opinion that epicutaneous allergen-specific immunotherapy is attracting increasing attention because of its capacity to offer a safe, needle-free and potentially self-administrable treatment option for IgE-mediated allergic diseases [43].

Advantages of the epidermis for allergen delivery are thought to be that it contains high numbers of potent antigen-presenting Langerhans cells to enhance efficacy as well as it being a nonvascularized multilayer epithelium in order to minimize inadvertent systemic distribution of the allergen and therefore systemic allergic side effects [44,45]. Whether allergen preparations for intralymphatic or epicutaneous delivery of allergen immunotherapy will be certified as registered products and will become commercially available in the future cannot be predicted thus far.

Do adjuvants change immunotherapy delivery methods?

MPL

The addition of adjuvants is aimed at reinforcing the immunological modulation of allergen-specific T-cell responses toward tolerance. Under this premise, in Europe a preparation containing monophosphoryl lipid A (MPL) is available. MPL is a derivative of lipid A from the lipopolysaccharide (LPS) of the cell wall of *Salmonella minnesota* R595. In the 1950s, the adjuvant properties of LPS were examined for the first time in animal studies for

vaccinations [46]. Subsequent modifications to the LPS molecule led to an almost complete elimination of the toxicity of MPL with obtained immunogenic properties [47]. Today, MPL-containing vaccines are available for various indications (hepatitis B, cervical cancer, and so on) [46]. In allergen-specific immunotherapy, MPL has been used as an adjuvant in an *in vitro* study by Puggioni *et al.* [47].

Mononuclear cells from 13 grass pollen allergic patients were incubated with an extract of phleum pratense with and without MPL. Th1 respectively Th2-associated cytokine production was analyzed and revealed an attenuation of the Th-1 directed immune response with a significant increase in IFN- γ production and significantly decreased IL-5 production in the costimulation of allergen and adjuvant in comparison to sole incubation with allergen.

The results of other *in vitro* studies of mononuclear cell cultures also demonstrated increased production of the Th1-associated cytokines IL-10 and IL-12, which was mediated by binding of MPL to TLR2 and TLR4 [46].

A Th-1-directed cytokine profile was also found in a placebo-controlled double-blind study of 14 patients with tree pollen allergy that received short-term preseasonal therapy with a MPL-adjuvanted allergoid [48]. There was significantly increased production of IFN- γ as well as suppression of Th2-related cytokines IL-4 and IL-5 in the season if compared with the placebo group.

In a larger DBPC Phase III trial on 141 grass pollen-allergic patients, the clinical efficacy of SCIT was described with a grass pollen allergoid using tyrosine as a depot carrier with MPL adjuvant [49].

Compared with placebo, in the first grass pollen season after a preseasonal short therapy with only four injections of either placebo or verum, a significantly improved nasal and conjunctival symptom score as well as a significantly improved combined symptom medication score was found. In addition, the actively treated patients had significantly reduced reactivity in the skin prick test. Immunologically, an increase in grass pollen-specific IgG and suppression of the seasonal IgE-increase ('blunting') was seen in the treatment group.

However, the previously published clinical studies on the use of MPL as an adjuvant in SCIT leave the question of whether the observed clinical and immunological effects are actually caused by the adjuvant due to a lack of efficacy trials in comparison to an allergen extract with and without adjuvant.

When using the sublingual route for delivery of allergen immunotherapy, adjuvants such as MPL may also be an interesting option. *In vitro* data by Allam *et al.* gave evidence that human oral dendritic cells express high levels of lipopolysaccharide receptors (CD14) as well as TLR4 receptors on their surface [50,51]. Later, laboratory experiments by Allam *et al.* found an enhanced IL-10 production and a suppression of T-cell proliferation when oral DCs were stimulated with TLR4 ligands [52].

Based on these findings, the authors group performed the first Phase I/II dose-ranging placebo-controlled clinical trial with MPL-adjuvanted SLIT on 80 grass-pollen allergic adult patients [53]. Patients were randomized into the following four therapy groups receiving different doses of a grass pollen extract and

different doses of MPL: group 1: 9.45 µg Phl p 1, no MPL; group 2: 9.45 µg Phl p 1 + 21 µg MPL; group 3: 9.45 µg Phl p 1 + 52.5 µg MPL; and group 4: 19 µg Phl p 1 + 52.5 µg MPL. Besides a good tolerability to all investigated MPL doses, patients receiving the highest amount of MPL revealed early immunological effects as well as the highest proportion of negative allergen-specific nasal challenge test results after initiation of a 2-month SLIT course, whereas the increase in the allergen dose had no such effect. These encouraging preliminary results on adjuvanted SLIT in humans should now be confirmed in larger clinical Phase II/III trials.

Aluminum hydroxide

In allergen-specific immunotherapy, aluminum hydroxide (AH) was introduced by Sledge in 1938 due to its marked deposit effect in order to minimize the release of the allergen and thus the rate of systemic unwanted reactions [54]. In addition, immunological effects of a costimulatory dose of allergens and AH have been shown, which indicate an adjuvant effect of this depot carrier. In animal experiments, AH has been shown to act as an adjuvant (review in [55]). In flow cytometric analyses, the Th2 cytokine-weighted (IL-5 and IL-13) cytokine pattern was significantly reduced after stimulation of sensitized mononuclear cells by the administration of AH [56], which may be a hint for a potential protolerogenic effect due to the increase of protolerogenic Treg cells and shift in 'Th2 to Th1 shift' balance. However, the specific adjuvant effect of AH in the immune response of SIT has not been found in more detail.

In a large, multicenter DBPC dose-finding study in 410 grass pollen allergic patients, the efficacy of a grass pollen extract bound to AH in two maximal doses (10,000 or 100,000 units SQ) has been examined [57]. Here, there were some dose-dependent effects in clinical symptoms and medication scores of actively treated patients during the grass pollen season compared with the placebo group.

In a recent randomized, controlled, parallel-group trial from the authors' group, patients with grass pollen induced rhinoconjunctivitis with/without asthma were treated with an optimized ratio of a grass pollen extract and adjuvant AH-SCIT formulation (AVANZ, ALK, Denmark) [58]. The trial included up-titration with five injections (300, 600, 3000, 6000 and 15,000 SQ+) injected in either weekly intervals (group 1) or intervals of 3–4 days (group 2) followed by two maintenance injections (15,000 SQ+) – approximately 10 weeks of treatment. The immunological effects (primary end point) and tolerability (secondary end point) of the up-titration schedules were evaluated. A total of 400 patients were treated (group 1: 201; group 2: 199). In both groups, an immunological response with statistically significant increases in levels of IgE-blocking factor, IgG(4) and IgE ($p < 0.001$) was induced from baseline to the end of the trial. The most frequently reported adverse events were local injection site reactions such as injection site swellings (group 1: 30% of patients; group 2: 41% of patients). Other frequently reported adverse events included systemic reactions (group 1: 21% of patients; group 2: 33% of patients), primarily mild-to-moderate allergic rhinitis and urticaria. The authors judged this new fast up-dosed immunologically enhanced SCIT with an optimized allergen/adjuvant ratio to induce significant immunological effects and to have an acceptable safety profile. Whether and to what extent AH has adjuvant actions

in addition to its storage function cannot be answered definitively, as appropriate 'head-to-head' comparison studies (allergen extract vs AH-adjuvant allergen extract) are still pending. The use of other molecules as adjuvants in SIT is currently under investigation in clinical trials (reviewed in [46]).

Immunomodulating substances

Virus-like particles & CpG-motifs

Virus-like particles (Vlps) are supramolecular particles with diameters in the range of 25–100 nm. Their structure is either icosahedral or rod-like [59]. A key attribute is their property to spontaneously self-assemble into virus-shaped particles. These self-assembly systems follow the expression of one or several viral proteins – mostly viral envelop proteins, sometimes viral core proteins [60]. Vlps consist of highly repetitive surfaces, a proteinaceous shell and a lumen in which certain adjuvants (pathogen-associated molecular patterns) can be stored [59]. These features make Vlps a potent vaccine platform enabling a rapid and fulminant humoral immune response. Thus, Vlps can be used as carriers displaying antigens of choice on the surface [60,61].

At the start of a sufficient immune reaction, Vlps are taken up by APCs. The size of Vlp nanoparticles (20–200 nm) then facilitates B-cell receptor crosslinking, B-cell activation and activation of complement, stimulation of CD4 T cells, crosspresentation by MHC class I molecules and priming of CD8 T-cell responses [62,63].

TLR ligand adjuvants: CpG motifs

Research has revealed that Vlps typically induce poor T-cell responses in the absence of stimulating adjuvants. By adding stimulating adjuvants, the innate immune system will be activated more easily, leading to an efficient long-lasting adaptive immune response [59,60]. TLR ligands were found to be stimulating adjuvants, which could be combined with Vlps or used alone [64,65].

They belong to the so-called pathogen-associated molecular patterns. One of the best analyzed and characterized TLR ligands are non-methylated CpG oligodeoxynucleotides (CpG-ODN/CpG-motifs). CpG-motifs interact with intercellular TLR9 localized in the endoplasmic reticulum mainly in plasmacytoid dendritic cells and B cells [64,65].

Actual clinical experience with Vlps/CpG-motifs for the treatment of allergic rhinitis

Lately, Vlp technology and CpG-motifs have been tested in various routes and combinations to treat different clinical disorders. The most successful Vlp representatives probably include the HPV vaccines Gardasil (Merck & Co, NJ, USA) and Cervarix (GlaxoSmithKline, London, UK), which have been licensed for the European market and partly for the US market [66,67]. Actual clinical experiences with Vlps/CpG-motifs for treatment of allergic rhinitis have been presented in four clinical studies.

Kündig *et al.* conducted a randomized Phase I study with Qβ Vlp coupled to a synthetic 16-amino acid sequence of Der p 1 (Qβ-Der p 1) which was applied to 24 healthy volunteers. The 24 healthy participants were allocated to four groups: group 1 received 50 µg Qβ-Der p 1 intramuscularly; group 2

received 50 µg Qβ-Der p 1 subcutaneously; group 3 received 10 µg Qβ-Der p 1 intramuscularly; and group 4 received 10 µg Qβ-Der p 1 subcutaneously. All subjects received another two injections of 10 µg (after 1 month and 3 months). Antibody titers were monitored for 18 months. Only mild local reactions were reported. Furthermore, rapid IgM and IgG responses (especially IgG 1 and IgG3) and highly specific anti-Der p1 and anti-Qβ antibodies (~30-fold) were induced. IgE antibodies did not change. No difference was observed between the intramuscular and subcutaneous application mode [68].

Creticos *et al.* tested an Amb a 1 antigen conjugated to CpG-ODN in a randomized, double-blind, placebo-controlled Phase II study in 25 patients allergic to ragweed. All patients were vaccinated six times at weekly intervals. The primary end point (nasal vascular permeability assessed by nasal-lavage albumin) could not be influenced significantly by the active treatment. Allergic rhinitis symptoms were relieved and quality-of-life scores were improved considerably during the first ragweed pollen season after treatment. The benefit also remained during the next pollen season (2 years after vaccination). No serious adverse events correlated to the verum treatment appeared. Specific IgE was diminished during ragweed season, but only a moderate Amb a 1-specific and ragweed-specific IgG response was induced [69].

Senti *et al.* investigated the safety, tolerability and clinical efficacy of A-type CpG-ODN packaged in Qβ Vlp (QβG10) together with a standardized house dust mite (HDM) extract. Twenty patients allergic to HDM were enrolled in a single-center, open-label study. Immunotherapy consisted of the following two phases: first, the patients were treated with sole allergen extract according to a standard dose-escalating cluster regimen, followed by immunotherapy with allergen and QβG10 given six times every 1–2 weeks. Clinical end points included conjunctival provocation test, allergic and asthmatic symptom and quality-of-life assessments, skin prick tests and antibody titers. The combination of QβG10 and HDM allergen extract was safe and well tolerated. Symptoms of allergic rhinitis and allergic asthma were significantly reduced and HDM-specific IgG antibodies increased. IgE antibodies declined after a slight increase at the beginning of therapy. The authors proposed that CpG-motifs might alleviate allergic symptoms alone due to the activation of the innate immune system and Th1 cells [70].

This might conform with the ‘hygiene hypothesis’, suggesting that the underlying reason for the rapidly increasing incidence of allergy is due to the reduced exposure to environmental pathogens [71–73]. One step to a normal immune response is suggested to be the shift from emphasized Th2 response to a Th1 response. However, it seems to be unlikely that the beneficial effects of microbial exposure (such as CpG-ODNs) result mainly from a ‘protective’ Th1 response. Moreover, the generation of T-regulatory cells seems to play a major role in the balance of Th1 and Th2 effector cells [72,73]. Thus, allergies might be treated by CpG-motifs. Questions remain on how CpG-ODNs would interact with the induction of T-regulatory cells and Th1, or which other cells are important for their influence on allergy.

Our group published a randomized, double-blind, placebo-controlled, multicenter Phase IIb study using a Qβ Vlp filled with an A-type-CpG-ODN (CYT003-QβG10) [74]. A total of 299 patients allergic to HDM were included and received six weekly injections with either study drug or placebo. Overall, the study lasted 9 weeks. Clinical end points were ocular and nasal allergy symptom medication score, quality-of-life score and conjunctival provocation tests. The treatment was found to be safe and generally well tolerated. Symptom medication scores were significantly improved. The interesting fact in this clinical trial was that CpG-ODNs seemed to influence allergic symptoms positively, even in the absence of a specific allergen.

Unsolved issues in Vlp technology include whether antigen-specific T-cell presentation is needed for induction of a balanced immune response and which types of antibodies have the most protective abilities. In addition, it might be helpful to understand more explicitly the interaction between Vlp and APCs or T-regulatory cells. This could solve the question of whether allergen components need to be attached to Vlp or not. Meanwhile, many types of recombinant hypoallergens or allergen peptides have been developed. Perhaps combined with Vlp technology, those antigens could evoke an even greater breakthrough for the treatment of allergic diseases. The first clinical experiences with different compositions of Vlp/CpG-motif vaccines proved to have good clinical tolerance and immunogenic potential. Larger studies have to follow on to gain more detailed knowledge of this new technology. Certainly, Vlp/CpG-motifs show great potential to be promising candidates for the prophylactic or therapeutic treatment of allergic rhinitis.

Expert commentary & five-year view

Allergen-specific immunotherapy (SIT) is a disease-modifying, immunologically based treatment option for IgE-mediated allergies such as allergic rhinoconjunctivitis and allergic asthma. From the different delivery options for SIT, subcutaneous and sublingual immunotherapy are well documented – at least on the level of certain well-documented products. Other local immunotherapies, such as bronchial, nasal and oral applications, are not accepted. SCIT and SLIT can affect the progression of allergic disease in a certain population of patients, sometimes permanently.

New application routes such as epicutaneous and intralymphatic delivery of allergens have been evaluated in clinical trials and may become more important. Moreover, new immune-modifying agents such as Vlp and CpG-motifs and adjuvants may dramatically change allergen immunotherapy in the future. Over the next 5 years, the authors anticipate that results from hypothesis-driven randomized controlled trials will become available that can hopefully clarify the clinical value of these immune-modifying agents.

With regard to the epicutaneous and intralymphatic immunotherapies, commercial extracts need to be developed that are based on the current European regulations for the classification, composition and quality requirements of allergen extracts. Therefore, clinical trials with allergen products for epicutaneous and intralymphatic immunotherapy needs to be performed that deliver data on gradual dose-escalation studies and DBPC efficacy trials.

Key issues

- Subcutaneous immunotherapy is the standard delivery method in allergen-specific immunotherapy.
- Sublingual immunotherapy is the second best evaluated delivery method that has been especially well documented for grass allergen tablets.
- Nasal, oral and bronchial immunotherapy are not accepted as delivery methods for allergen-specific immunotherapy.
- Intralymphatic and epicutaneous immunotherapy are experimental methods for allergen immunotherapy that need further evaluation in larger clinical trials.
- Virus-like particles and CpG-motifs, as well as adjuvants such as monophosphoryl lipid A, may change allergen immunotherapy in the future.

References

- 1 Asher MI, Montefort S, Björkstén B *et al.*; ISAAC Phase Three Study Group. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet* 368(9537), 733–743 (2006).
- 2 Björkstén B, Clayton T, Ellwood P, Stewart A, Strachan D; ISAAC Phase III Study Group. Worldwide time trends for symptoms of rhinitis and conjunctivitis: Phase III of the International Study of Asthma and Allergies in Childhood. *Pediatr. Allergy Immunol.* 19(2), 110–124 (2008).
- 3 *Weißbuch Allergie in Deutschland*. Ring J, Bachert C, Bauer CP, Czech W (Eds). Urban & Vogel, Munich, Germany (2009).
- 4 Maurer M, Zuberbier T. Undertreatment of rhinitis symptoms in Europe: findings from a cross-sectional questionnaire survey. *Allergy* 62(9), 1057–1063 (2007).
- 5 Jacobsen L, Niggemann B, Dreborg S *et al.*; The PAT investigator group. Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study. *Allergy* 62(8), 943–948 (2007).
- 6 Calderon MA, Gerth van Wijk R, Eichler I *et al.*; European Academy of Allergy and Clinical Immunology. Perspectives on allergen-specific immunotherapy in childhood: an EAACI position statement. *Pediatr. Allergy Immunol.* 23(4), 300–306 (2012).
- 7 Zuberbier T, Lotvall J. Allergies have a socioeconomic impact: a model calculation. *Allergy* 63(Suppl. 88), 612–621 (2008).
- 8 von der Schulenburg JM, Greiner W, Molitor S *et al.* [Kosten der Asthmatherapie nach Schweregrad: eine empirische Untersuchung.] *Med. Klin. (Munich)* 91, S670–S676 (1996).
- 9 Calderon MA, Alves B, Jacobson M, Hurwitz B, Sheikh A, Durham S. Allergen injection immunotherapy for seasonal allergic rhinitis. *Cochrane Database Syst. Rev.* 1, CD001936 (2007).
- 10 Radulovic S, Calderon MA, Wilson D, Durham S. Sublingual immunotherapy for allergic rhinitis. *Cochrane Database Syst. Rev.* 12, CD002893 (2010).
- 11 Abramson MJ, Puy RM, Weiner JM. Injection allergen immunotherapy for asthma. *Cochrane Database Syst. Rev.* 8, CD001186 (2010).
- 12 Kleine-Tebbe J, Bufer A, Ebner C *et al.*; Deutschen Gesellschaft für Allergologie und klinische Immunologie; Ärzteverbandes Deutscher Allergologen; Gesellschaft für Pädiatrische Allergologie und Umweltmedizin; Österreichischen Gesellschaft für Allergologie und Immunologie; Schweizerischen Gesellschaft für Allergologie und Immunologie. [Die spezifische Immuntherapie (Hypsensibilisierung) bei IgE-vermittelten allergischen Erkrankungen]. *Allergo J.* 18, 508–537 (2009).
- 13 Calderon MA, Larenas D, Kleine-Tebbe J *et al.* European Academy of Allergy and Clinical Immunology task force report on 'dose–response relationship in allergen-specific immunotherapy'. *Allergy* 66(10), 1345–1359 (2011).
- 14 Malling HJ, Abreu-Nogueira J, Alvarez-Cuesta E *et al.* Local immunotherapy. *Allergy* 53(10), 933–944 (1998).
- 15 Zuberbier T, Bachert C, Bousquet PJ *et al.* GA² LEN/EAACI pocket guide for allergen-specific immunotherapy for allergic rhinitis and asthma. *Allergy* 65(12), 1525–1530 (2010).
- 16 Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J. Allergy Clin. Immunol.* 102(4 Pt 1), 558–562 (1998).
- 17 Canonica GW, Bousquet J, Casale T *et al.* Sublingual immunotherapy: World Allergy Organization position paper 2009. *Allergy* 64(Suppl. 91), 1–59 (2009).
- 18 Walker SM, Durham SR, Till SJ *et al.*; British Society for Allergy and Clinical Immunology. Immunotherapy for allergic rhinitis. *Clin. Exp. Allergy* 41(9), 1177–1200 (2011).
- 19 Matricardi PM, Kuna P, Panetta V, Wahn U, Narkus A. Subcutaneous immunotherapy and pharmacotherapy in seasonal allergic rhinitis: a comparison based on meta-analyses. *J. Allergy Clin. Immunol.* 128(4), 791–799.e6 (2011).
- 20 Calderon MA, Cox L, Casale TB, Moingeon P, Demoly P. Multiple-allergen and single-allergen immunotherapy strategies in polysensitized patients: looking at the published evidence. *J. Allergy Clin. Immunol.* 129(4), 929–934 (2012).
- 21 Eng PA, Borer-Reinhold M, Heijnen IA, Gnehm HP. Twelve-year follow-up after discontinuation of preseasonal grass pollen immunotherapy in childhood. *Allergy* 61(2), 198–201 (2006).
- 22 Fujita H, Soyka MB, Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy. *Clin. Transl. Allergy* 2(1), 2 (2012).
- 23 James LK, Shami MH, Walker SM *et al.* Long-term tolerance after allergen immunotherapy is accompanied by selective persistence of blocking antibodies. *J. Allergy Clin. Immunol.* 127, 509–516 (2011).
- 24 Haugaard L, Dahl R, Jacobsen L. A controlled dose–response study of immunotherapy with standardized, partially purified extract of house dust mite: clinical efficacy and side effects. *J. Allergy Clin. Immunol.* 91(3), 709–722 (1993).
- 25 Didier A, Malling HJ, Worm M *et al.* Optimal dose, efficacy, and safety of once-daily sublingual immunotherapy with a 5-grass pollen tablet for seasonal allergic rhinitis. *J. Allergy Clin. Immunol.* 120(6), 1338–1345 (2007).
- 26 Chapman MD, Ferreira F, Villalba M *et al.*; CREATE consortium. The European Union CREATE project: a model for

- international standardization of allergy diagnostics and vaccines. *J. Allergy Clin. Immunol.* 122(5), 882–889.e2 (2008).
- 27 Corrigan CJ, Kettner J, Doerner C, Cromwell O, Narkus A; Study Group. Efficacy and safety of preseasonal-specific immunotherapy with an aluminium-adsorbed six-grass pollen allergoid. *Allergy* 60(6), 801–807 (2005).
 - 28 DuBuske LM, Frew AJ, Horak F *et al.* Ultrashort-specific immunotherapy successfully treats seasonal allergic rhinoconjunctivitis to grass pollen. *Allergy Asthma Proc.* 32(3), 239–247 (2011).
 - 29 Pfaar O, Urry Z, Robinson DS *et al.* A randomized placebo-controlled trial of rush preseasonal depigmented polymerized grass pollen immunotherapy. *Allergy* 67(2), 272–279 (2012).
 - 30 Pfaar O, Robinson DS, Sager A, Emuzyte R. Immunotherapy with depigmented-polymerized mixed tree pollen extract: a clinical trial and responder analysis. *Allergy* 65(12), 1614–1621 (2010).
 - 31 Zielen S, Kardos P, Madonini E. Steroid-sparing effects with allergen-specific immunotherapy in children with asthma: a randomized controlled trial. *J. Allergy Clin. Immunol.* 126(5), 942–949 (2010).
 - 32 Riechelmann H, Schmutzhard J, van der Werf JF, Distler A, Kleinjans HA. Efficacy and safety of a glutaraldehyde-modified house dust mite extract in allergic rhinitis. *Am. J. Rhinol. Allergy* 24(5), e104–e109 (2010).
 - 33 Durham SR, Emminger W, Kapp A *et al.* SQ-standardized sublingual grass immunotherapy: confirmation of disease modification 2 years after 3 years of treatment in a randomized trial. *J. Allergy Clin. Immunol.* 129(3), 717–725.e5 (2012).
 - 34 Ott H, Sieber J, Brehler R *et al.* Efficacy of grass pollen sublingual immunotherapy for three consecutive seasons and after cessation of treatment: the ECRIT study. *Allergy* 64(9), 1394–1401 (2009).
 - 35 Valovirta E, Berstad AK, de Blic J *et al.*; GAP investigators. Design and recruitment for the GAP trial, investigating the preventive effect on asthma development of an SQ-standardized grass allergy immunotherapy tablet in children with grass pollen-induced allergic rhinoconjunctivitis. *Clin. Ther.* 33(10), 1537–1546 (2011).
 - 36 Horak F, Ziegelmayer P, Ziegelmayer R *et al.* Early onset of action of a 5-grass-pollen 300-IR sublingual immunotherapy tablet evaluated in an allergen challenge chamber. *J. Allergy Clin. Immunol.* 124(3), 471–477.e1 (2009).
 - 37 Senti G, Prinz Vavricka BM, Erdmann I *et al.* Intralymphatic allergen administration renders specific immunotherapy faster and safer: a randomized controlled trial. *Proc. Natl Acad. Sci. USA* 105(46), 17908–17912 (2008).
 - 38 Senti G, Graf N, Haug S *et al.* Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy. *J. Allergy Clin. Immunol.* 124(5), 997–1002 (2009).
 - 39 Senti G, Cramer R, Kuster D *et al.* Intralymphatic immunotherapy for cat allergy induces tolerance after only 3 injections. *J. Allergy Clin. Immunol.* 129(5), 1290–1296 (2012).
 - 40 Kündig TM. Immunotherapy concepts under investigation. *Allergy* 66(Suppl. 95), 60–62 (2011).
 - 41 Senti G, Johansen P, Kündig TM. Intralymphatic immunotherapy: from the rationale to human applications. *Curr. Top. Microbiol. Immunol.* 352, 71–84 (2011).
 - 42 Senti G, von Moos S, Tay F *et al.* Epicutaneous allergen-specific immunotherapy ameliorates grass pollen-induced rhinoconjunctivitis: a double-blind, placebo-controlled dose escalation study. *J. Allergy Clin. Immunol.* 129(1), 128–135 (2012).
 - 43 von Moos S, Kündig TM, Senti G. Novel administration routes for allergen-specific immunotherapy: a review of intralymphatic and epicutaneous allergen-specific immunotherapy. *Immunol. Allergy Clin. North Am.* 31(2), 391–406, xi (2011).
 - 44 Senti G, von Moos S, Kündig TM. Epicutaneous allergen administration: is this the future of allergen-specific immunotherapy? *Allergy* 66(6), 798–809 (2011).
 - 45 Senti G, Freiburghaus AU, Kündig TM. Epicutaneous/transcutaneous allergen-specific immunotherapy: rationale and clinical trials. *Curr. Opin. Allergy Clin. Immunol.* 10(6), 582–586 (2010).
 - 46 Pfaar O, Böttcher I, Klimek L. Modulation of specific immunotherapy by adjuvants: immunological effects and clinical perspectives. *Allergo J.* 18, 261–266 (2009).
 - 47 Puggioni F, Durham SR, Francis JN. Monophosphoryl lipid A (MPL) promotes allergen-induced immune deviation in favour of Th1 responses. *Allergy* 60(5), 678–684 (2005).
 - 48 Stuck BA, Schneider-Gene S, Klimek L, Schäfer D, Hörmann K. Short-term immunotherapy with birch pollen allergoid plus monophosphoryl lipid A (MPL) influences cytokine production of peripheral T cells in patients with allergic rhinitis. *ACI International* 16, 60–64 (2004).
 - 49 Drachenberg KJ, Wheeler AW, Stuebner P, Horak F. A well-tolerated grass pollen-specific allergy vaccine containing a novel adjuvant, monophosphoryl lipid A, reduces allergic symptoms after only four preseasonal injections. *Allergy* 56(6), 498–505 (2001).
 - 50 Allam JP, Novak N, Fuchs C *et al.* Characterization of dendritic cells from human oral mucosa: a new Langerhans' cell type with high constitutive FcεpsilonRI expression. *J. Allergy Clin. Immunol.* 112(1), 141–148 (2003).
 - 51 Novak N, Bieber T, Allam JP. Immunological mechanisms of sublingual allergen-specific immunotherapy. *Allergy* 66(6), 733–739 (2011).
 - 52 Allam JP, Peng WM, Appel T *et al.* Toll-like receptor 4 ligation enforces tolerogenic properties of oral mucosal Langerhans cells. *J. Allergy Clin. Immunol.* 121(2), 368–374.e1 (2008).
 - 53 Pfaar O, Barth C, Jaschke C, Hörmann K, Klimek L. Sublingual allergen-specific immunotherapy adjuvanted with monophosphoryl lipid A: a Phase I/IIa study. *Int. Arch. Allergy Immunol.* 154(4), 336–344 (2011).
 - 54 Sledge RF. Treatment of hay-fever with alum-precipitated pollen. *US Naval Med. Bulletin* 36, 18 (1938).
 - 55 Rask C, Lund L, Lund G *et al.* An alternative allergen:adjuvant formulation potentiates the immunogenicity and reduces allergenicity of a novel subcutaneous immunotherapy product for treatment of grass-pollen allergy. *Clin. Exp. Allergy* 42(9), 1356–1368 (2012).
 - 56 Wilcock LK, Francis JN, Durham SR. Aluminium hydroxide down-regulates T helper 2 responses by allergen-stimulated human peripheral blood mononuclear cells. *Clin. Exp. Allergy* 34(9), 1373–1378 (2004).
 - 57 Frew AJ, Powell RJ, Corrigan CJ, Durham SR; UK Immunotherapy Study Group. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. *J. Allergy Clin. Immunol.* 117(2), 319–325 (2006).
 - 58 Pfaar O, Jung K, Wolf H *et al.* Immunological effects and tolerability of a new fast up dosed immunologically enhanced subcutaneous immunotherapy formulation with optimized allergen/adjuvant ratio. *Allergy* 67(5), 630–637 (2012).

- 59 Bachmann MF, Jennings GT. Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. *Nat. Rev. Immunol.* 10(11), 787–796 (2010).
- 60 Jennings GT, Bachmann MF. The coming of age of virus-like particle vaccines. *Biol. Chem.* 389(5), 521–536 (2008).
- 61 Jennings GT, Bachmann MF. Designing recombinant vaccines with viral properties: a rational approach to more effective vaccines. *Curr. Mol. Med.* 7(2), 143–155 (2007).
- 62 Yewdell JW. Designing CD8⁺ T cell vaccines: it's not rocket science (yet). *Curr. Opin. Immunol.* 22(3), 402–410 (2010).
- 63 Tewalt EF, Grant JM, Granger EL *et al.* Viral sequestration of antigen subverts cross presentation to CD8⁺ T cells. *PLoS Pathog.* 5(5), e1000457 (2009).
- 64 Klinman DM. Immunotherapeutic uses of CpG oligodeoxynucleotides. *Nat. Rev. Immunol.* 4(4), 249–258 (2004).
- 65 Bode C, Zhao G, Steinhagen F, Kinjo T, Klinman DM. CpG DNA as a vaccine adjuvant. *Expert Rev. Vaccines* 10(4), 499–511 (2011).
- 66 Garland SM, Hernandez-Avila M, Wheeler CM *et al.*; Females United to Unilaterally Reduce Endo/Ectocervical Disease (FUTURE) I Investigators. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. *N. Engl. J. Med.* 356(19), 1928–1943 (2007).
- 67 Paavonen J, Jenkins D, Bosch FX *et al.*; HPV PATRICIA study group. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a Phase III double-blind, randomised controlled trial. *Lancet* 369(9580), 2161–2170 (2007).
- 68 Kündig TM, Senti G, Schnetzler G *et al.* Der p 1 peptide on virus-like particles is safe and highly immunogenic in healthy adults. *J. Allergy Clin. Immunol.* 117(6), 1470–1476 (2006).
- 69 Creticos PS, Schroeder JT, Hamilton RG *et al.*; Immune Tolerance Network Group. Immunotherapy with a ragweed-Toll-like receptor 9 agonist vaccine for allergic rhinitis. *N. Engl. J. Med.* 355(14), 1445–1455 (2006).
- 70 Senti G, Johansen P, Haug S *et al.* Use of A-type CpG oligodeoxynucleotides as an adjuvant in allergen-specific immunotherapy in humans: a Phase I/IIa clinical trial. *Clin. Exp. Allergy* 39(4), 562–570 (2009).
- 71 Stelmach I, Smejda K, Jerzynska J *et al.* Decreased markers of atopy in children with presumed early exposure to allergens, unhygienic conditions, and infections. *Ann. Allergy Asthma Immunol.* 99(2), 170–177 (2007).
- 72 Braun-Fahrlander C, Riedler J, Herz U *et al.*; Allergy and Endotoxin Study Team. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N. Engl. J. Med.* 347(12), 869–877 (2002).
- 73 Riedler J, Braun-Fahrlander C, Eder W *et al.*; ALEX Study Team. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 358(9288), 1129–1133 (2001).
- 74 Klimek L, Willers J, Hammann-Haenni A *et al.* Assessment of clinical efficacy of CYT003-QbG10 in patients with allergic rhinoconjunctivitis: a Phase IIb study. *Clin. Exp. Allergy* 41(9), 1305–1312 (2011).

Websites

- 101 FORSA Umfrage 2012. www.derma.de/fileadmin/eingang/FORSA_Allergien.de
- 102 Guideline on allergen products: production and quality issues. Committee for Medicinal Products for Human Use. www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003333.pdf
- 103 Guideline on the clinical development of products for specific immunotherapy for the treatment of allergic diseases. Committee for Medicinal Products for Human Use. www.emea.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003605.pdf
- 104 Paul-Ehrlich-Institut. List of approved allergen therapies. www.pei.de/cdn_236/nn_154892/DE/arzneimittel/allergene/therapieallergene/therapieallergene-node.html?__nnn=true



A comparison of immunotherapy delivery methods for allergen immunotherapy

To obtain credit, you should first read the journal article. After reading the article, you should be able to answer the following, related, multiple-choice questions. To complete the questions (with a minimum 70% passing score) and earn continuing medical education (CME) credit, please go to www.medscape.org/journal/clinicalimmunol. Credit cannot be obtained for tests completed on paper, although you may use the worksheet below to keep a record of your answers. You must be a registered user on Medscape.org. If you are not registered on Medscape.org, please click on the New Users: Free Registration link on the left hand side of the website to register. Only one answer is correct for each question. Once you successfully answer all post-test questions you will be able to view and/or print your certificate. For questions regarding the content of this activity, contact the accredited provider, CME@medscape.net. For technical assistance, contact CME@webmd.net. American Medical Association's Physician's Recognition Award (AMA PRA) credits are accepted in the US as evidence of participation in CME activities. For further information on this award, please refer to <http://www.ama-assn.org/ama/pub/category/2922.html>. The AMA has determined

that physicians not licensed in the US who participate in this CME activity are eligible for **AMA PRA Category 1 Credits™**. Through agreements that the AMA has made with agencies in some countries, AMA PRA credit may be acceptable as evidence of participation in CME activities. If you are not licensed in the US, please complete the questions online, print the AMA PRA CME credit certificate and present it to your national medical association for review.

Activity Evaluation

Where 1 is strongly disagree and 5 is strongly agree

	1	2	3	4	5
1. The activity supported the learning objectives.					
2. The material was organized clearly for learning to occur.					
3. The content learned from this activity will impact my practice.					
4. The activity was presented objectively and free of commercial bias.					

1. You are seeing a 14-year-old young man with a history of moderate persistent asthma and allergic rhinitis. What should you consider regarding the epidemiology of these diseases?

- ☐ A Up to two-thirds of adolescents may report rhinitis symptoms
- ☐ B Allergic rhinoconjunctivitis affects approximately 5% of the general population
- ☐ C Allergic asthma affects approximately 1% of the general population
- ☐ D Most patients with allergy use prescription medications to alleviate symptoms

2. After further questioning, you realize that this patient is a candidate for immunotherapy. What should you consider regarding the practice of subcutaneous immunotherapy (SCIT)?

- ☐ A It is better to wait until this patient is older to begin SCIT
- ☐ B The treatment course with allergoids is usually 20 weeks
- ☐ C Treatment can be provided throughout the year or just seasonally
- ☐ D Antihistamines should be avoided around the time of SCIT

3. The patient initially refuses SCIT. Which of the following means of delivery of immunotherapy is most accepted after SCIT?

- ☐ A Transbronchial
- ☐ B Sublingual
- ☐ C Epicutaneous
- ☐ D Nasal

4. After some discussion, the patient agrees to SCIT. What should you consider regarding adjuvants which may enhance the efficacy and tolerability of SCIT?

- ☐ A Monophosphoryl lipid A (MPL) acts mainly by increasing the expression of interleukin-5 (IL-5)
- ☐ B There is no clinical data to support the use of MPL in immunotherapy
- ☐ C Virus-like particles (VLPs) can self-assemble into virus-shaped particles
- ☐ D VLPs have yet to be incorporated into vaccines