

New routes of allergen immunotherapy

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ABSTRACT

Objectives: Allergen immunotherapy is the only cure for immunoglobulin E mediated type I respiratory allergies. Subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT) are the most common treatments. In this article, we reviewed new routes of allergen immunotherapy.

Methods: Data on alternative routes to allow intralymphatic immunotherapy (ILIT), epicutaneous immunotherapy (EPIT), local nasal immunotherapy (LNIT), oral immunotherapy (OIT), and oral mucosal immunotherapy (OMIT) were gathered from the literature and were discussed.

Results: ILIT features direct injection of allergens into lymph nodes. ILIT may be clinically effective after only a few injections and induces allergen-specific immunoglobulin G, similarly to SCIT. A limitation of ILIT is that intralymphatic injections are required. EPIT features allergen administration by using patches mounted on the skin. EPIT seeks to target epidermal antigen-presenting Langerhans cells rather than mast cells or the vasculature; this should reduce both local and systemic adverse effects. LNIT involves the spraying of allergen extracts into the nasal cavity. Natural or chemically modified allergens (the latter, termed allergoids, lack immunoglobulin E reactivity) are prepared in a soluble form. OIT involves the regular administration of small amounts of a food allergen by mouth and commences with low oral doses, which are then increased as tolerance develops. OMIT seeks to deliver allergenic proteins to an expanded population of Langerhans cells in the mucosa of the oral cavity.

Conclusions: ILIT, EPIT, LNIT, OIT, and OMIT are new routes for allergen immunotherapy. They are safe and effective.

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Allergen immunotherapy is the only cure for immunoglobulin E (IgE) mediated type I respiratory allergies.^{1,2} Subcutaneous immunotherapy (SCIT), which often serves as the reference therapy, has transformed allergy treatment. SCIT improves symptoms (asthma and rhinitis) and quality of life; it involves repetitive allergen administration and is associated with risks of severe systemic adverse effects, including anaphylaxis. Sublingual immunotherapy (SLIT) is a valid and noninvasive alternative, and is safe and efficacious when used to treat respiratory allergies.¹

Allergen-specific immunotherapy effectively treats allergic rhinitis. Allergens are commonly administered *via* repeated subcutaneous injections or as daily sublingual tablets taken for 3–5 years.³ At present, SLIT is the only validated noninvasive alternative to SCIT. SLIT is safe and efficacious when used to develop allergen-specific tolerance, as revealed by a double-blind, placebo-controlled phase III study that used large cohorts of adult and pediatric patients allergic to grass pollen or to house-dust mite (HDM).¹ Several new routes are currently under investigation: the intralymphatic, epicutaneous, intranasal, oral, and oral mucosal routes.^{1,4,5} SLIT exploits the tolerogenic environment of the oral mucosa. Epicutaneous allergen immunotherapy (EPIT) uses the immune cells of the epidermis to transport antigens to afferent lymph nodes, thus activating immune responses.⁶ Oral immunotherapy (OIT) has effectively induced both immunomodulation and “desensitization” (*i.e.*, an increase in the amount of food allergen that can be consumed as long as regular [typically daily] doses are continued). However, the ability of OIT to induce perma-

nent tolerance (*i.e.*, tolerance that persists after ingestion of the food ceases) seems to be limited. In addition, the short- and long-term safety of OIT is often poorly reported, which raises concerns about implementation in routine practice.⁷ In this article, the new routes for immunotherapy were discussed in detail.

Intralymphatic Immunotherapy

Intralymphatic immunotherapy (ILIT) was originally tested in the 1970s in the context of cancer immunotherapy.⁸ It was hoped that injection of antigens into a lymph node would enhance the development of protective immunity.^{9,10} The clinical trials conducted to date have shown that ILIT may be clinically effective after only a few injections and induces allergen-specific IgG as effectively as does SCIT.^{11,12} In an attempt to further improve ILIT, a recombinant allergen fused to a modular transporter antigen was prepared and shown to also stimulate regulatory T cells.¹² It remains to be seen whether ILIT is more effective than SCIT. An obvious limitation of ILIT is that intralymphatic injections are required.¹⁰ ILIT was previously used to enhance protective immunity against viruses and tumors; the vaccines were based on proteins, peptides, naked DNA, or tumor cells.^{13–15} ILIT is now being extensively investigated; it seems from clinical trials that the immunization protocol may be reduced (in terms of time) compared with that of SCIT, and that the allergen doses could be reduced 100–1000-fold without affecting efficacy (*i.e.*, allergen challenge was better tolerated than is the case with SCIT).¹⁶

Senti *et al.*,¹⁷ in a randomized double-blind trial, showed that ILIT was safe and induced tolerance to MAT-Fel d-1 (a cat antigen) delivered in alum after only three injections. Mo¹⁸ and Hylander *et al.*¹⁹ showed that ILIT by using grass- or birch-pollen extracts reduced nasal allergic symptoms without any safety problems, which indicated that ILIT might be less time consuming and more cost effective than SCIT. Several studies treatment duration is explored.³ Intralymph node injection delivers more allergen to dendritic cells to T and B cells. Local reactions are reduced; lymph nodes contain only a few mast cells.²⁰

A comparative clinical trial with 165 patients with grass pollen allergy showed that ILIT was comparable with SCIT in terms of efficacy, even when very low doses of allergens were used.¹¹ In this study, the patients were randomized to receive either SCIT with pollen extract over 3 years (the cumulative allergen dose was 4 million standardized quality units) or ILIT (three intralymphatic in-

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jections over 2 months; the cumulative allergen dose was 3000 standardized quality units). ILIT improved hay fever symptoms, reduced skin-prick test reactivity, and enhanced patient quality of life and compliance. Only mild adverse effects (local pain and inflammation) were noted.¹¹

Hylander *et al.*¹⁹ reported that direct intralymphatic injection afforded rapid beneficial outcomes with considerably lower doses of allergen (compared with SCIT) delivered *via* a markedly reduced number of injections. Patients with allergic rhinitis were given three intralymphatic inguinal injections of ALK Alutard (ALK-Abelló, Hørsholm, Denmark) (which contained 1000 subcutaneous units of birch or grass pollen), or placebo (the ALK diluent; ALK-Abelló). Test patients exhibited initial increases in allergen-specific IgE levels and the extent of peripheral T-cell activation. Clinical improvements in nasal allergic symptoms on challenge were evident, as were decreases in the nasal inflammatory responses. In addition, patients self-reported improvements in seasonal allergic disease. No such changes were evident in the placebo group. It was concluded that ILIT might represent a new paradigm in allergen-specific immunotherapy, from economic, social, and health care perspectives. Compared with SCIT, ILIT requires fewer treatment visits and injections, and considerably lower doses of allergen.

Hellkvist *et al.*³ studied 60 patients, during the period 2012–2014, who had moderate-to-severe allergies to birch pollen and mild-to-moderate allergies to grass pollen. Patients were randomized (in equal numbers) to receive three ultrasound-guided intralymphatic injections of placebo or ALK (Alutard Birch and Grass, 1000 standardized quality units) in the groin at 1-month intervals. Data on 36 patients had been analyzed to date for the period 2012–2013. There were 14 test patients and 22 patients who received placebo. All adverse effects were mild; 8% of test patients experienced local swelling at the injection site. In the placebo group, the quality of life scores worsened during the peak birch pollen season. This was not the case in the test group, in which the Specific Birch IgG4 levels increased 2 weeks after ILIT commenced; the Specific Birch IgE levels increased at 6–9 months. T-helper (Th) type 1 cell (CCR5⁺ CD4⁺ cell) numbers increased in the blood and lymph nodes of the test patients, as did blood memory T-cell numbers. It was concluded that ILIT that simultaneously targeted birch and grass pollen was safe and improved quality of life during the pollen season. This supports the idea that ILIT is an effective alternative form of immunotherapy. ILIT has yielded promising clinical results; thus, it is an attractive new approach for potentially reducing the treatment duration from years to weeks.¹

EPIT

Several recent attempts have been made to administer allergens *via* the skin. EPIT delivers allergens by using patches applied onto the skin. Several differed methods are under testing, and some data from animal studies and clinical trials are already available.^{21–24} Although some evidence of clinical effects has been presented, further data on the immunologic mechanism involved and measurable improvements in clinical parameters are required. In addition, it is not known whether EPIT induces allergen-specific IgG.¹⁰ However, interest in EPIT is growing, because it is a safe, noninvasive, patient-friendly treatment for IgE-mediated allergic diseases caused by either respiratory or food allergens.¹ By targeting epidermal antigen-presenting Langerhans cells, but neither mast cells nor the vasculature, EPIT should reduce both local and systemic adverse effects.²⁰ Some encouraging data are available.²⁵ Senti *et al.*²⁵ found that one 6-week course of EPIT, which used grass pollen, reduced symptom scores by >24% during the following pollen season; EPIT was convenient, safe, and efficacious.¹⁸

EPIT was used to deliver allergens of grass pollen, the HDM, or food (cow's milk, eggs, and peanuts) to intact or tape-stripped skin. Amelioration of nasal symptoms (triggered by grass pollen) was evident, and the treatment was well tolerated, with only minor local

eczematous reactions (when grass pollen and cow's milk allergens were used). No immunologic changes were evident in humans, but airway hyperresponsiveness, the numbers of eosinophils in bronchoalveolar lavage fluid, and the extent of Th2 responses (to ovalbumin, grass pollen, the HDM, and peanuts) were reduced in mice.^{21,26–30}

Senti *et al.*²² reported the results of a phase I/II study on the safety and efficacy of EPIT in patients allergic to grass pollen (*i.e.*, a *Phleum pratense* extract). Allergic symptoms were somewhat reduced on nasal provocation testing but were not significantly lower in the test group than in the placebo group. No serious adverse event was recorded; a few local eczematous reactions were noted, probably attributable to tape stripping of the skin before EPIT, which may induce the release of proinflammatory cytokines (*i.e.*, interleukin [IL]) 1, IL-6, IL-8, or tumor necrosis factor α) by keratinocytes. A new epicutaneous delivery system (Viaskin; DBV Technology, Bagneux, France) is currently being tested in humans and features a condensation chamber that releases soluble allergens while hydrating the epidermal layers. In a pilot EPIT study on children with allergies to cow's milk, the treatment was well tolerated. However, neither clinical efficacy nor any immunologic change (*i.e.*, a decrease in the milk-specific IgE level of cows) has yet been documented.³¹ EPIT is safe and well tolerated, which thus paves the way for further investigation of the efficacy of this treatment in large cohorts of patients with respiratory or food allergies. The allergen dose, number of doses required, and duration of treatment all require further investigation.¹

Local Nasal Immunotherapy

Local nasal immunotherapy (LNIT) involves the spraying of allergen extracts directly into the nasal cavity; the allergens, termed allergoids (and lacking IgE reactivity), are in a soluble form and are natural or chemically modified. LNIT afforded significant reductions in both the symptoms of, and medications required by, patients allergic to ragweed and grass pollens.^{32–35} The slight adverse effects were largely confined to the sites of administration.^{34,36} LNIT vaccines based on micronized, powdered allergen extracts of *Parietaria*, birch, and grass, or by using allergen-coated strips (*e.g.*, of the HDM allergen), enhanced clinical efficacy, and reduced local adverse effects.^{30,37–41} A 4-year course of LNIT afforded long-lasting relief of nasal symptoms.^{42,43} Recently, LNIT with rBet-v-1 reduced nasal flow in patients allergic to birch pollen.³² Interestingly, the hypoallergenic rBet-v-1 fragments had no effect on symptom scores, which indicates that an IgE epitope is essential for induction of allergen-specific tolerance.⁴⁴

LNIT has been used to deliver allergens of pollens (grasses, ragweed, *Parietaria*, and birch) as well as the HDM allergen. The allergens tested were natural extracts, allergoids, macronized powders, and strips coated with extracts. Improved clinical efficacy and long-lasting protective effects were noted in patients with rhinitis triggered by grass, birch, ragweed, *Parietaria* pollens, and the HDM allergen. Inflammatory infiltration and intercellular adhesion molecule 1 expression were reduced at local sites (the *Parietaria* allergen); IgA, IgE, and IgG levels rose (the ragweed and grass antigens); airway hyperresponsiveness was reduced; eosinophil numbers in bronchoalveolar lavage fluid fell; and the Th2 responses were reduced. Th1/regulatory T cells were induced, and saliva IgA levels were increased in murine models.^{30,32–52} LNIT seems to be a valuable treatment for allergic rhinitis, and safe and efficient. However, compliance may be a problem; frequent nasal sprays are required. Also, it is difficult to precisely control the dose of allergen administered.¹

OIT

The prevalence of food allergy is increasing worldwide. The U.S. Centers for Disease Control reported that such allergies have increased by 18% over the past 2 decades.⁵³ The prevalence of peanut allergy has doubled in recent years and now affects 1–2% of young

children in the United States, United Kingdom, Canada, and Australia.⁵⁴ In parallel, food-induced anaphylaxis is also increasing.⁵⁵ Currently, the only treatment for a food allergy is strict dietary avoidance; thus, therapies are urgently needed. Promising therapies under investigation include both allergen-specific and nonspecific approaches.⁵⁶ Not surprisingly, the therapies focus on foods that most frequently induce severe anaphylactic reactions (peanuts, tree nuts, and shellfish) and on the most common food allergens, such as cow's milk and eggs.

An approach that is attracting significant public, media, and scientific interest is OIT,⁵⁷ which was first used in the early 20th century to treat a young boy with a severe anaphylactic allergy to eggs.⁵⁸ OIT is the subject of many ongoing studies. Because food allergies most likely result from failed development of oral tolerance or the breakdown of normal systems (after a gastrointestinal infection or sensitization to a cross-reactive allergen *via* inhalation), the oral route of administration exploits the cells and immune pathways involved in induction of oral tolerance. Results of animal studies indicate that high-dose feeding of an antigen triggers a state of nonresponsiveness attributable to either anergy or the deletion of antigen-specific T lymphocytes; continuous low-dose ingestion may induce protective suppressive responses by regulatory T cells.⁵⁹ In contrast, intermittent feeding or nonoral exposure (*e.g.*, cutaneous or inhalational) may induce IgE sensitization and cause allergic symptoms to develop on food ingestion.⁶⁰

The desensitized state, defined as the ability to ingest a substantial amount of food of a special allergen-free diet with protection against any severe reaction to accidental allergen exposure, is achieved by ~50–75% of children treated with OIT. The frequency of permanent tolerance is unknown, but OIT of longer duration may afford this permanent tolerance. Adverse effects are common both during initial dose escalation and home dosing. Most reactions are mild (oral pruritus, abdominal discomfort, and rashes) and reduce in frequency as OIT continues. Severe reactions (which require epinephrine treatment) have been reported during home dosing. Factors associated with an increased risk of a reaction to a previously tolerated dose during home dosing include exercise, a viral infection, dosing on an empty stomach, menstruation, and exacerbation of asthma.⁶¹

OIT involves the regular administration of small amounts of allergen (food) by mouth. Desensitization (the ability to tolerate an allergen while on immunotherapy) is rapidly induced, and then, over time, tolerance (the long-term ability to consume a food allergen safely after immunotherapy is discontinued) develops. Patients who undergo OIT generally ingest a protein powder in water or another liquid vehicle, *e.g.*, apple sauce. Treatments are typically commenced in a controlled setting (*e.g.*, office, hospital clinic), in which gradually increasing doses of allergen are given up to a target dose. After this, standard protocols indicate that most dosing can be done at home, with doses increased on a weekly basis in the office. There is overwhelming evidence that OIT induces desensitization such that the patient will be able to ingest or be exposed to the food without any reaction. OIT should theoretically cure any IgE-based food allergy.⁶²

OIT begins with administration of low oral doses, which are increased as tolerated. OIT has successfully desensitized patients allergic to different foods, including eggs, milk, peanuts, and tree nuts.^{63–65} The usual goal of therapy is to reduce or eliminate the severity of the reactions that follow accidental ingestion, which means that only relatively low oral maintenance doses must be tolerated; however, occasionally, the goal of OIT has been to induce tolerance to much greater maintenance (dietary) doses of the food, but no consensus has yet emerged about the best protocol in terms of dosing schedule and dose timing. Currently, OIT is still considered to be an experimental technique, associated with significant concerns in terms of safety and the long-term outcomes.⁶⁶

Although OIT can effectively increase the amount of food that can be tolerated by an individual with food allergy, allergic reactions, including anaphylaxis, are frequently observed during desensitization. For example, >90% of patients undergoing oral milk OIT develop reactions, and 10–20% require epinephrine at some point dur-

ing desensitization.⁶⁷ Results of a recent meta-analysis of OIT indicated that the frequent and serious adverse reactions might outweigh any benefits.^{66,68,69} Moreover, because the frequent allergic reactions prevent dose increases, it takes a median of 20–60 weeks to reach a maintenance dose, and the highest oral dose achieved is often below the target maintenance dose. Importantly, as many as 10–30% of patients with food allergy are refractory to desensitization, particularly those with higher initial food-specific IgE levels.^{70–74} Given these safety issues, OIT is currently performed primarily in academic centers, and most experts strongly believe that it should not be recommended for use in the community.^{66,75}

The possible mechanisms of oral desensitization include increased production of food-specific IgG4, a decrease in the levels of food-specific IgE antibodies, and reduction in the extent of activation of mast cells and basophils. In contrast, when permanent oral tolerance is established, food may be ingested without triggering allergic symptoms, despite a previous period of abstinence. Development of persistent tolerance likely requires changes in the activities of regulatory T cells and an immunologic deviation away from the proallergic Th2 response. The permanence of protection may be tested by intentional interruption of dosing for at least 4 weeks, followed by supervised oral food challenge.⁷⁶ It is currently unclear whether permanent tolerance is a function of OIT duration. In other words, will such tolerance be achieved by any individual with allergy if the duration of OIT is sufficiently long? It remains possible that some individuals with allergy can never become truly tolerant.

A recent study provided more insight into the mechanism of peanut OIT, and, presumably, these findings may also be relevant to other allergens.⁷⁷ Of 29 children with peanut allergies who completed the protocol, 27 ingested 3.9 g of peanut protein during the final food challenge. Most symptoms noted during OIT resolved spontaneously or on administration of antihistamines. Six months later, titrated skin-prick test data and the extent of basophil activation had significantly decreased. Peanut-specific IgE antibody concentrations decrease in 12–18 months, whereas the IgG4 levels increased significantly. Serum factors inhibited formation of IgE-peanut complexes in an IgE-facilitated allergen-binding assay. The extent of secretion of the proallergic cytokines IL-10, IL-5, interferon γ , and tumor necrosis factor α from peripheral blood mononuclear cells increased over a period of 6–12 months. Peanut-specific forkhead box protein-3-positive regulatory T cells increased in number up to 12 months and decreased thereafter. In addition, T-cell microarrays revealed down-regulation of genes involved in the apoptotic pathway.⁷⁷

Oral Mucosal Immunotherapy

Oral mucosal immunotherapy (OMIT) was developed to bring allergenic proteins in contact with a wider surface area of oral mucosa and a larger population of oral Langerhans cells than afforded by OIT. The oral Langerhans cells express the high-affinity IgE receptor, thus efficiently binding allergens and exhibiting the natural pro-tolerogenic characteristics required for successful immunotherapy.^{78,79} The lowest density of oral Langerhans cells is in the sublingual region, whereas the highest density, the most active expression of the high-affinity receptor, and the lowest densities of eosinophils and mast cells are found in other areas of the oral cavity, including the vestibule and buccal mucosa.^{80,81} In a mouse model, Zhang *et al.*⁸² discovered that, in contrast to the buccal mucosa, repeated antigen painting of the sublingual mucosa rapidly (within 24 hours) depleted submucosal dendritic cells, and the extent of subsequent recruitment was also markedly lower. In a 2013 position paper, the World Allergy Organization stated that “targeting the vestibule with allergen vaccine with or without adjuvant has the potential to induce enhanced immune deviation or tolerance, possibly with a lower potential for mast cell-related local side effects...”⁸³

One case series indicated that preseasonal and coseasonal OMIT was useful to treat spring allergies; a special toothpaste that contains protein extracts from birch and oak improved Allergy Outcome Sur-

vey scores and decreased skin reactivity.⁸⁴ More recently, an open-label study that used a liquid preparation reported similar improvements after OMIT (compared with SLIT) in both the combined and quality of life scores.⁸⁵ The adherence rates were 80% in the OMIT group and 62% in the SLIT group; significant rises in specific IgG4 levels were evident in both groups during the first 6 months of treatment. Burks *et al.*⁸⁶ indicated that a combination of OMIT with adjuvants that reduce food allergies might be a safe method of progressing from SLIT to OIT, particularly in individuals at high risk. In a recent study on personal treatment preferences, individuals with chronic rhinitis who underwent allergy testing ranked OMIT, SLIT, and allergen immunotherapy as their first choices, at equal frequencies; SCIT was significantly less preferred.⁸⁷

CONCLUSION

Although SCIT and SLIT have been successfully used by clinicians for many years, significant recent advances have been made in alternative allergen delivery routes when food or airborne allergies are to be treated. The examples given in this article included ILIT, EPIT, LNIT, OIT, and OMIT. The goals of these delivery systems are not only to improve efficacy and limit adverse events but also to create treatments that fit easily into daily life. If individuals with allergy have options that they can integrate into their lifestyles, they will be more likely to adhere to the 3–5 years of consistent therapy required to achieve long-lasting results.

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