Human Vaccines & Immunotherapeutics 8:10, 1525-1533; October 2012; © 2012 Landes Bioscience

New routes for allergen immunotherapy

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Keywords: allergy, immunotherapy, administration route, intralymphatic, epicutaneous, transcutaneous, clinical trials

Abbreviations: SIT, allergen specific immunotherapy; ILIT, intralymphatic immunotherapy; EPIT, epicutaneous immunotherapy; SLIT, sublingual immunotherapy; QoL, quality of life; DC, dendritic cells; LCs, Langerhans cells; APCs, Antigen presenting cells

IgE-mediated allergy is a highly prevalent disease in the industrialised world. Allergen-specific immunotherapy (SIT) should be the preferred treatment, as it has long lasting protective effects and can stop the progression of the disease. However, few allergic patients choose to undergo SIT, due to the long treatment time and potential allergic adverse events. Since the beneficial effects of SIT are mediated by antigen presenting cells inducing Th1, Treg and antibody responses, whereas the adverse events are caused by mast cells and basophils, the therapeutic window of SIT may be widened by targeting tissues rich in antigen presenting cells. Lymph nodes and the epidermis contain high densities of dendritic cells and low numbers of mast cells and basophils. The epidermis has the added benefit of not being vascularized thereby reducing the chances of anaphylactic shock due to leakage of allergen. Hence, both these tissues represent highly promising routes for SIT and are the focus of discussion in this review.

Introduction

Allergic rhino-conjunctivitis affects more than 20% of the population in western Europe,[1] and represents a significant cause of illness with impact on daily activities and sleep quality.[2] While many patients respond effectively to symptomatic pharmacotherapy such as antihistamines and corticosteroids, a substantial proportion of patients report inadequate symptom alleviation and reduced quality of life (QoL).[3] Here, allergen-specific immunotherapy (SIT) offers a disease-specific causative treatment alternative by inducing tolerance to the allergen.[4] Hence, patients who suffer from allergies in which allergen avoidance is not possible, such as pollen or house dust mite allergy, patients with systemic reactions to hymenoptera venom and patients whose symptoms have not responded adequately to optimal pharmacotherapy are usually the prime candidates for SIT.[5]

Until recently, SIT was predominantly administered subcutaneously (SCIT) as described in the early works by Noon and Freeman.[6,7] SCIT comprises repeated administration of allergen extracts, of as many as 50–80 injections over a period of at least three years, but for immunotherapy to be effective, careful patient selection is required.[8,9] Provided adequate precautions are taken, SIT is safe,[10] but a decision whether to treat with immunotherapy will depend on a variety of factors, and conventional SCIT has a low appeal for most patients. Only approximately 5% of patients with allergic rhino-conjunctivitis choose to undergo immunotherapy, due to the long treatment duration with severe impact on daily life, e.g., numerous doctor visits with consequential absence from school or work. Personal and organizational factors then also determine whether one type of immunotherapy is more suitable than another, e.g., SCIT or the more patient-friendly, but equally long and less validated sublingual immunotherapy (SLIT), which can be self-administered. In response to the drawbacks of SCIT and SLIT, current SIT research in allergy focuses on developing methods that are more patient-friendly in order to facilitate better patient compliance.[4,5,11-13]

One of the major challenges in this field of research is to balance efficacy and safety of the new SIT methods. Immunologically, SIT and conventional vaccination do not substantially differ.[14] In conventional childhood vaccination, three injections of antigen and adjuvant are typically sufficient to induce long-term protection against vaccine-preventable diseases caused by microbes and higher doses typically produce stronger immune responses. From a qualitative viewpoint, it would be beneficial to use vaccines that are able to shift the type of immune response from allergy-associated T-helper type 2 (Th2) toward the more protective Th1 and Treg- based immune responses. This could be achieved by using higher doses of allergen with stronger Th1-stimulating adjuvants. However, in conventional SIT, high doses of antigen cannot be used since patients are allergic to the antigen used. Therefore, antigen doses in conventional SIT must be kept low to reduce the risk of patients suffering from local side effects and systemic and serious adverse effects. Hence, the goal of current SIT research is to allow strong stimulation of the immune system, while bypassing potential adverse reactions.

Besides using hypo-allergenic and recombinant allergens, one strategy to improve the therapeutic window of SIT is to choose administration routes that allow efficient targeting of potent professional antigen presenting cells (APCs), while avoiding tissues with high density of mast cells. Moreover, SIT in highly vascularized tissues should also be avoided in order to avoid vascular

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Submitted: 07/05/12; Revised: 08/15/12; Accepted: 08/23/12
http://dx.doi.org/10.4161/hv.21948

Table 1. Clinical trials with allergen-specific intralymphatic (ILIT) or epicutaneous (EPIT) immunotherapy

	Allergen, adjuvant	Trial type	Patient numbers	Reference
ILIT	Bee venom, extract, Alum	Open label	8	Senti and Kündig in preparation
	Bee venom, extract, Alum	Multicenter RCT	67	Senti and Kündig in preparation
	Grass pollen, extract, Alum	DB RCT	20	Senti et al.[135]
	Recombinant Fel d1, Alum	DB RCT	58	Senti et al.[139]
	Grass pollen, extract, Alum	DB RCT	45	Malling et al.[136]
EPIT	Grass pollen, patch	DB RCT	37	Senti et al.[54]
	Grass pollen, patch	DB RCT	30 (Children)	Agostinis et al.[69]
	Grass pollen, patch	DB RCT	132	Senti et al.[68]
	Grass pollen, patch	DB RCT	98	Senti and Kündig in preparation
	Cow milk allergy, Viaskin® patch	DB RCT	19 (Children)	Dupont et al.[45]
	Peanut allergy, Viaskin® patch	DB RCT	110	NCT01170286

Abbreviations: RCT, randomized controlled trial; DB RCT, double blinded randomized controlled trial; Alum, aluminum hydroxide.

leakage of allergen, which can cause systemic allergic reactions or anaphylaxis. Two tissues which fit the above-described properties are the epidermal layer of the skin and the lymph nodes; the skin being the largest and most accessible organ system and the lymph nodes, the organs with the highest density of immune competent cells as well as being the final target of any vaccine antigen or SIT allergen. During the last decade, we and others have studied the use of these routes for vaccination and immunotherapy (**Table 1**) and in this review, we will discuss the outcomes of the research with a special focus on allergen-specific immunotherapy.

Epicutaneous Immunotherapy (EPIT)

The skin as an immunological organ in topical vaccination. Briefly, the human skin consists of the epidermis and the underlying dermis. Although the epidermis of the palms and soles are thicker by 1 mm, it typically forms a 50–150 µm thick mechanical barrier with a 15–20 µm thick outer layer of cornified keratinocytes in a lipid-rich matrix known as the *stratum corneum*, which effectively excludes the entry of large molecules.[15-17] While predominantly consisting of keratinocytes, the epidermis also contains specialized cells such as pigment-producing melanocytes and antigen-presenting Langerhans cells (LCs).[16] LCs account for only 3–5% of the epidermal cells, but are attractive targets for vaccines as they cover up to 20% of the skin surface by forming a network with their dendrites.[15,18] The non-vascularized epidermis is separated by the basement membrane from the more complex dermis, which harbors a great diversity of cells as well as lymphatic and vascular conduits. Besides fibroblasts, macrophages and mast cells, the dermis comprises different subsets of dermal dendritic cells (DCs), as well as, depending on the inflammatory stage, other immune cells of myeloid or lymphoid origin.[16,19]

The skin with its associated lymphoid tissue (SALT) is considered an immunologically competent organ system.[20] Although many of the immunologic properties of the skin are known,[21] specific functions of the different DC subsets and the keratinocytes with regard to the stimulation and shaping of adaptive immune response remains to be unravelled.[22,23] Whereas epidermal LCs have been demonstrated to hold a key role in elicitation of CD8+ T-cell responses and in shaping Th2-type/Treg-type responses, activation of dermal DC subsets has been found to be essential for B cell class switching and induction of a Th1-type response.[24,25] In line with this, dermal DC subsets were observed to preferentially localize to the B-cell areas of draining lymph nodes, whereas LCs were observed to migrate to the T-cell areas.[26] While previously considered mostly immunologically inactive and only forming part of the physical barrier function of the skin, keratinocytes have now been recognized to play a pivotal role in triggering and guiding the adaptive immune responses.[23,27] These epithelial cells express different molecular mediators under different conditions, thereby governing dictating a variety of responses. A slight stress to the epithelium, such as abrasion, may trigger secretion of the IL-7-like cytokine thymic stromal lymphopoietin (TSLP), IL-25 and IL-33, which in turn instruct non-inflammatory Treg- or Th2-type responses.[28-32] On the other hand, epithelial damages may trigger expression of additional molecules such as IL-1α, IL-6 and TNF-α, which skew Th1-type responses.[27] The degree of epithelial damage might explain the observed dichotomy of different DC subsets: while mild irritations induce non-inflammatory responses transmitted by LCs, stronger epithelial damages induce pro-inflammatory response performed by dermal DCs. The exact understanding of these processes would certainly support a more rational design of vaccines for epi- or transcutaneous administration.

Although the skin is easily accessible, topical application of a vaccine in a cream or in a patch does not typically induce an immune response because of the low permeability of vaccines through the *stratum corneum*. When, in ancient times, the Indians first used the skin to vaccinate against smallpox, the *stratum corneum* was disrupted by scratching, also called scarification.[33] Today, this has been replaced by tape stripping and other abrasive methods,[34,35] which aim at gently removing the cornified keratinocytes without disrupting the underlying epidermal layers. Similarly, but more precisely, APCs of the epidermis can nowadays be directly targeted by using microneedle arrays with defined needle lengths thanks to latest technological progress.[15,36-42] These methods are associated with physical irritation of keratinocytes, leading to secretion of cytokines, which in turn

facilitates activation of the immune system.[27,43] Alternatively, penetration of antigens through the epidermis can be enhanced by skin hydration over a time period of at least 4 to 10 h.[44] This is typically achieved by application of an occlusive patch leading to sweat accumulation.[45,46]

Allergen-specific immunotherapy by topical epicutaneous administration. Despite the ancient success of epicutaneous smallpox vaccination,[47] this route of administration did not attract major attention until the end of the 20th century, when Glenn and coworkers demonstrated efficacious transcutaneous vaccination against numerous infectious diseases as well as cancer.[33,34,48-53] A decade later, our group published the first placebo controlled trial on allergen-specific epicutaneous immunotherapy (EPIT).[54]

However, the first records describing the administration of allergens via the skin date back to 1929. Soon after the introduction of SCIT by Leonard Noon,[6] the risk of suffering from anaphylaxis and other allergic adverse events upon subcutaneous allergen administration was recognized as a real problem[55] and spurred testing of intradermal pollen extract administrations.[56] Similar to the intradermal allergy testing of today, pollen extracts were applied to the skin. The authors reported that the treatment was safe and highly successful with symptom amelioration in all the 29 patients after administration of three doses.

Another event that suggested the potential suitability of EPIT was an observation reported in 1921, when an asthmatic patient experienced complete symptom relief after administration of horse dander on scarificated skin, a method called "cutiréactions répétées."[57] Then, French allergologists paved the way for EPIT during the 1950s and 60s. Pautrizel and coworkers[58] administered allergen extracts onto epidermis that was slightly rubbed, while the team of Blamoutier[59,60] applied allergen drops onto heavily scarified skin, a method called "méthode de quadrillage cutané." Both groups observed high treatment success, but while the former method required a large number of applications the method of Blamoutier could be performed co-seasonally with significant symptom amelioration after an average of four treatments. Both methods were associated with fewer and milder allergic side effects than typically observed with SCIT.[58-60] Several confirmatory trials were performed in the following years by the Swiss clinicians Eichenberger and Storck, who reported a treatment success rate of approximately 80%.[61] Symptoms rapidly diminished after a single application, and the effect lasted from three days to three weeks. Hence, 6 to 12 treatments were required for a symptom free season and the method was reported to be also effective in patients who responded inadequately to conventional SCIT.[61-63]

Inspired by these early reports, we performed the first randomized placebo-controlled study that tested the clinical efficacy and safety of EPIT in patients allergic to grass pollen.[54] Twelve patches with allergen or placebo were applied on the upper arm, before and during the pollen season, and the application area was prepared by tape stripping.[64-67] A 70% improvement of hay fever symptoms in the treated patients was observed, while the placebo effect accounted for 20%. No severe systemic allergic reactions were observed, but local eczematous skin reactions were frequently reported.

The first study was followed up with another phase I/IIa trial with 132 patients in order to define optimal allergen doses.[68] Hay-fever symptoms during the pollen season were reduced by more than 30% in the first year and by 24% in the second year in the high-dose group when compared with those in the placebo group. The alleviation of symptoms in the follow-up year was dependent on the treatment dose, but higher allergen doses were associated with more local adverse events (AEs), manifested by pruritus, erythema, wheal or eczema. Eleven (8.3%) systemic AEs of grades 1 to 2 required treatment and led to study exclusion. No drug-related serious AE was recorded.

A third placebo-controlled trial, including 98 patients, was initiated in 2009 and recently completed (NCT00777374). The goal of this study was to further compare the efficacy of EPIT using the combined symptom medication score as well measuring immunological responses. Results are expected in 2013. In support of our results, Agostinis and coworkers recently demonstrated efficacy and safety of EPIT when used as a treatment for grass pollen allergy in children.[69] Hay fever symptoms as well as antihistamine use were significantly reduced in the active treatment group.

Furthermore, the French company DBV Technologies currently develops an occlusive patch for EPIT. It is known that the atopy patch test can induce an eczematous skin reaction even on intact skin.[70] This suggests efficient antigen penetration, and DBV Technologies has confirmed this with an occlusive patch initially developed for diagnostic purposes (Diallertest).[45,46,71] Known commercially as Viaskin®, this epidermal delivery system (EDS) relies on the ability to deliver whole protein molecules to the skin, facilitated through hydration of the *stratum corneum* under an occlusive chamber. Whole protein, delivered via such a system, has been demonstrated to accumulate in the *stratum corneum*, from where it diffuses down to immune cells in the viable layers of the epidermis.[46,71,72] Murine studies, designed to test therapeutic efficacy of the Viaskin® EDS, demonstrated equivalence of EPIT and SCIT[46] as well as EPIT and SLIT[73] in preventing allergic airway reactions upon airway allergen challenge. Moreover, EPIT using the Viaskin® EDS was also efficient in the treatment of food allergy as measured by prevention of mast cell degranulation upon oral allergen challenge.[71,74] A clinical pilot trial was launched to test efficacy and safety of Viaskin® EDS EPIT in children with allergy to cow milk. The treatment was well tolerated with no serious systemic allergic reaction, but there was a significant increase in local eczematous reactions.[45] A trend toward greater tolerance to cow milk was observed in the treated group upon food provocation. Phase I (NCT01170286) and phase II trials (NCT01197053) have been initiated to substantiate these findings when using Viaskin® EDS for treatment against peanut allergy.

Intralymphatic Immunotherapy

Immunological specialties of the lymph nodes. T- and B-cell receptors are randomly rearranged to generate a large repertoire of lymphocytes that can specifically recognize all possible antigens. Conversely, however, the frequencies of antigen-specific

lymphocytes are so low (approximately one in ten million) that antigens must be presented to millions of T and B cells in order to find their match and to elicit a response. Therefore, antigens need to either drain into secondary lymphatic organs or be transported there. Here, antigens can be presented to high numbers of T and B cells for stimulation of an immune response.[75] Antigens staying outside of secondary lymphoid organs have little chance to encounter specific T or B cells and are therefore poorly immunogenic.[76-78]

Lymph vessels drain substances from the interstitial fluid into regional lymph nodes with highly variable efficiency. As lymph vessels have evolved to drain pathogens into lymph nodes, viruses and small particles (20–200 nm) drain freely and efficiently from the peripheral sites into lymph nodes. However, only a low percentage of the particles drain into the lymph nodes.[79] Larger bacteria and particles (500–2,000 nm) are mostly transported into lymph nodes by DCs and other APCs. On the other hand, drainage of non-particulate antigens from the periphery to lymph nodes is typically less efficient and only one thousandth to one millionth of the antigen dose reaches the lymph nodes. Because many of the currently used vaccines are non-particulate, one may expect that the direct administration of a vaccine into a lymph node would increase antigen presentation and stimulation of immune responses.

Intralymphatic immunotherapy. A large number of studies, both pre-clinical and clinical have been based on the intralymphatic administration of drug or vaccines. In humans, the drug or vaccine preparations are typically administered intralymphatically into an inguinal lymph node under ultrasound control, a procedure that is uncomplicated and performed routinely by sonologists as well as by radiologists for the administration of imaging agents. For a comprehensive review, see references 12 and 80–83.

Antigen pulsed DCs injected into the lymph node localize to the paracortex.[84-86] As DCs and T cells are present at very high densities in lymph nodes, co-stimulatory signals for T- and B-cell induction may be provided as a bystander effect. While some clinical trials using intranodal therapy with DCs have suggested enhanced immune responses,[87,88] other trials failed to demonstrate advantage of intranodal over intradermal DCs delivery.[84,89] We also found that non-professional APCs, such as a fibro-sarcoma cell line efficiently induced antigen specific CD8+ T-cell responses in lymph nodes via direct antigen presentation on the MHC class I molecule present on the fibro-sarcoma.[76,90] Intranodal therapy with tumor cells has been tried in both human cancer patients and dogs with indication of success.[91-95]

Direct administration of MHC class I binding peptide vaccines into lymph nodes or the spleen has been demonstrated to enhance CD8 T-cell responses that were protective against viral challenge and tumor growth in mice.[96] Similarly, intranodal immunisation also dramatically enhanced the efficacy of plasmid DNA vaccination in mice[97-99] as well as RNA vaccination.[100-102]

Intranodal immunisation with proteins for induction of antibodies was performed surprisingly early. At a time when it was difficult to purify high quantities of proteins, researchers were looking for a more efficient route of immunisation.[103] By intralymphatic immunotherapy, only nanograms of proteins were required to elicit sufficiently strong immune responses.[104] Likewise, targeted lymph node administration is also extensively documented to be the most efficient way to immunize macaques against SIV.[105] Similar results were obtained in macaques vaccinated with other proteins.[106-111]

Several clinical trials with intranodal therapy have confirmed these initial pre-clinical studies. Most of these studies have been performed in cancer patients usually as autologous vaccination using antigen-pulsed DCs.[88,112-118] This treatment was typically safe and stimulated antigen-specific and cytotoxic CD8 T-cell responses in the patients. Although intralymphatic immunotherapy with DCs has been associated with improved survival,[112] increased immune responses were not always followed by marked clinical benefits.[115] In other cancer trials, the intranodal vaccines were based on plasmid DNA[119] or plasmid DNA and peptide in a prime-boost regime.[120,121] Finally, intranodal injections of vaccines based on viral vectors have been studied in melanoma patients, who responded with strong cytotoxic and other immunological T-cell responses as well as some clinical benefits.[122,123]

Allergen-specific intralymphatic immunotherapy (ILIT). As mentioned above, IgE-mediated allergies, such as allergic rhinoconjunctivitis and asthma, have become highly prevalent[124-127] and the gold standard of therapy is subcutaneous allergen-specific immunotherapy (SCIT), which confers long-term benefits, and which interrupts with the atopic march that is the expansion of allergic sensitizations to other allergens[128] and the progression from allergic rhinitis to asthma.[129] From a medical perspective, SCIT is therefore superior to symptomatic treatments, but its efficacy should be improved and the treatment time reduced to make SIT more patient friendly.

Therefore, we have investigated whether SCIT could be enhanced by intralymphatic administration of allergens. In mice, we have demonstrated that ILIT with a variety of allergens, e.g., bee venom phospholipase-A2, ovalbumin and allergen extracts from grass pollen, birch pollen and cat dander, stimulated anti-allergic and protective B- and T-cell immune responses.[130-134] In fact, changing from the subcutaneous route to direct intralymphatic injection significantly enhanced the efficacy of immunization, inducing allergen-specific IgG2a antibody responses that were 10–20 times higher with only 0.1% of the allergen dose. Since allergic side effects are proportional to the allergen dose, intralymphatic SIT should also have the potential to reduce side effects, as lower doses of allergens are required for efficacious SIT. Moreover, allergen ILIT enhanced IL-1, IFN-γ, IL-4 and IL-10 secretion when compared with subcutaneous SIT, suggesting that ILIT did not polarize the immune response allergen toward Th1, Th2 or Tregs, but generated overall stronger responses.

Studies of biodistribution in mice showed that approximately 100-fold higher allergen doses reached the lymph nodes after intralymphatic than after subcutaneous injection in the same draining region.[132] Similar results were obtained in humans when intralymphatic injections were compared with subcutaneous injection using radio tracing.[81,83] Essentially, when the same dose of a 99mTc labeled protein was injected into either a superficial inguinal lymph node or subcutaneously at a site just

10 cm above the contralateral inguinal lymph node, only a small fraction of the subcutaneously administered protein reached the lymph nodes after 4 h and this fraction did not increase after 24 h. However, within 20 min of the intralymphatic injection, the protein drained into the deep subcutaneous lymph nodes and even further into a pelvic lymph node. These experiments clearly demonstrated the potential of allergen ILIT to that of allergen SCIT.

Clinical trials with allergen ILIT. In a first clinical trial, eight patients with bee-venom allergy grade III, who would have received approximately 70 subcutaneous injections of each 100 μg bee venom extract under normal SCIT protocol, received three injections of only 10 μg directly into the inguinal lymph node. In this proof of concept trial, seven out of the eight treated patients were protected against a subsequent bee sting challenge. In a larger multicenter clinical trial conducted after the pilot study with 66 grade III and IV bee venom allergic patients, allergen-ILIT-mediated protection was shown to correlate with immunological parameters (Senti et al., manuscript in preparation). The safety and efficacy of intranodal bee venom allergen extract (Bee AlleVax) was further tested in a multicenter study. Between 2001 and 2003, 67 subjects from 15 centers across Europe and in Australia were randomized to receive either 10 or 20 μg doses of Bee AlleVax™ via intranodal injections. Clinical endpoints included bee venom specific IgE and IgG serum levels and the degree of protection after a bee sting challenge. Desensitization by ILIT not only resulted in increased IgG and IgE levels, but also in a high number of serious adverse events occurring both after ILIT and after bee sting challenge. For this reason, the study was terminated by the sponsors. The high number of serious adverse events may suggest that the immunological protection offered by Bee AlleVax™ was insufficient. Two confounding factors may explain the failure of Bee AlleVax™. First, a major fraction of the bee venom extract remained un-absorbed to the alum (personal communication from S.J. McCormack, former CEO of AlleCure Corp.). This may cause vascular leakage of free allergen. Second, the bee venom allergen extract was prepared in US using American honey bees and the studies were done on patients from Europe and Australia, and it is known that continental differences in the content of bee venom allergens exists.

In another Swiss trial, 165 patients with grass pollen-induced rhino-conjunctivitis were randomized to receive either 54 subcutaneous injections with pollen extract over three years [cumulative allergen dose 4,031,540 standardized quality units (SQ-U)] or three intralymphatic injections over two months (cumulative allergen dose 3,000 SQ-U); the trial was monocentric and open labeled.[135] Patients were evaluated after four months, one year and three years. Three low-dose intralymphatic allergen administrations increased tolerance upon nasal provocation with pollen within four months after treatment. Tolerance was long lasting and equivalent to that achieved after standard SCIT. Allergen ILIT ameliorated hay fever symptoms, reduced skin prick test reactivity, decreased specific IgE in serum and caused fewer adverse events than did SCIT. ILIT also enhanced compliance and was less painful than venous puncture. In conclusion, intralymphatic allergen administration enhanced safety and efficacy of immunotherapy and could reduce treatment time from three years to eight weeks.

In a recently reported Danish clinical trial on ILIT in grass pollen allergic patients by Malling and coworkers, three and six low dose intralymphatic injections were found to induce IgG4, but in contrast to the described Swiss trial, no reduction in hay fever symptoms could be found.[136] Hay fever symptoms actually worsened when compared with placebo, and IgE was boosted after allergen ILIT. In the Danish trial, intralymphatic grass pollen extract injections were given on a weekly interval, and not monthly, as in the Swiss trial.[135] Experience from vaccine immunology recommends a time interval between priming injections of at least three weeks to allow development of successive waves of antigen specific primary responses and affinity maturation of memory B cells.[137] Also, the continued antigen presentation generated by weekly administration in the Danish trial by Malling and coworkers may have favored boosting of Th2 responses, as has been previously demonstrated in mouse models.[138] With the on-and-off allergen profile generated by monthly injections in the Swiss trial, no boosting of IgE was observed.[135]

Recently, we applied ILIT using a MHC class II-targeting cat dander allergen; a recombinant Fel d 1 conjugated to a TAT translocation sequence and invariant chain (MAT-Fel d 1). In this randomized, placebo-controlled and double-blind trial, ILIT with MAT-Fel d 1 in alum was compared with ILIT with placebo in cat dander allergic patients.[139] Three monthly injections with MAT-Fel d 1 elicited no adverse events, and there was significant increase in allergen tolerance after nasal provocation. In addition, allergen ILIT stimulated regulatory T-cell responses with IL-10 cytokine secretion and increased cat dander-specific IgG4 production.

Conclusions

The ideal route for allergen-specific immunotherapy is one with a high density of potent professional APCs, so that immunogenicity of the allergen is increased enabling dose-reduction as well as reduction of the number of allergen administrations. Also the ideal route of allergen immunotherapy should contain only few mast cells to avoid local allergic reactions, and vascularization should be poor to minimize the risk of allergen leakage or inadvertent intravascular allergen administration entailing severe anaphylactic side effects. Both, the epidermis and the lymph node contain a high density of DCs. While lymph nodes contain very few mast cells, the epidermis contains none. Further advantages of the epidermis as route of allergen administration are the accessibility and fact that the epithelium is not vascularized.

It has been verified in clinical trials that both safety and efficacy of intranodal SIT (ILIT) is improved when compared with conventional subcutaneous SIT (SCIT). For epicutaneous SIT (EPIT), clinical comparisons with SCIT have not yet been done because current guidelines for testing efficacy of SIT require placebo control instead of SCIT control. The type of allergens suitable for ILIT and EPIT are in principle the same as those suitable

for SCIT. However, from a safety viewpoint, SIT with allergens that have very high affinity to IgE would surely benefit from ILIT as ILIT enables effective therapy with much lower doses than those required in effective SCIT. In conclusion, both the lymph node and the epidermis represent highly interesting routes for allergen immunotherapy, combining the advantages of high density of APCs and low frequency of mast cells and few vascular structures, factors that enable dose reduction, reduction of doctor visits and side effects.

Disclosure of Potential Conflicts of Interest

T.M.K. and G.S. are named as inventors on a patent on epicutaneous immunotherapy, and T.M.K. is named as the inventor on a patent on intralymphatic immunotherapy. Both patents are owned by the University of Zurich. P.J., S.M. and D.M. declare to have no relevant conflicts of interest.

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