



AIT: New Avenues in Allergen Immunotherapy

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Abstract

During the last decades a substantial increase of allergic diseases has been noticed including allergic asthma and rhinoconjunctivitis as well as food allergies. Since efficient avoidance of airborne – and often hidden – food allergens is not possible, allergen immunotherapy (AIT) is the only causative treatment with the goal of inducing allergen tolerance in affected individuals. Efficacy as well as safety of AIT significantly depends on how the allergen is presented to the immune system, meaning both the route and the form of its application. Here, new ways of allergen administration have lately been explored, some of which are auspicious candidates for successful implementation in the therapeutic management of immediate-type allergies. While the first oral AIT has been approved recently by the FDA for the treatment of peanut allergy, further interesting routes of allergen application include either epicutaneous, intradermal, intranasal, or intralymphatic delivery. Besides, rather the immunologically relevant peptides

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instead of whole allergen may be administered to develop tolerance. In this chapter, we will describe these new and promising avenues of allergen application in the field of AIT. In addition, we will discuss their potential for future treatment of IgE-mediated allergic diseases enhancing therapeutic efficiency while further minimizing the risks of adverse events.

Keywords

Allergen immunotherapy · Allergy · EPIT · ILIT · OIT

Abbreviations

AIT	Allergen immunotherapy
APC	Antigen-presenting cell
Breg cells	Regulatory B cells
EPIT	Epicutaneous immunotherapy
IDIT	Intradermal immunotherapy
ILIT	Intralymphatic immunotherapy
LNIT	Local nasal immunotherapy
OIT	Oral immunotherapy
SCIT	Subcutaneous immunotherapy
SLIT	Sublingual immunotherapy
Th cell	T helper cell
Treg cells	Regulatory T cells

Allergen immunotherapy (AIT) describes the establishment of immune – and hereby clinical – tolerance to an allergen, achieved by different cellular and humoral immune responses. Mechanisms involved in the induction of tolerance are, e.g., the promotion of both regulatory T (Treg) and B (Breg) cells, a diminished T helper (Th) 2 cell reactivity and the production of allergen-blocking IgG antibodies (Shamji and Durham 2017; Wambre 2015; Möbs et al. 2008). This requires successful delivery of allergen to antigen-presenting cells (APC) capable of initiating an immunoregulatory cross-talk between various T and B cell subsets. Depending on the specific circumstances of how and where to the allergen is applied, it can be recognized and taken up by different APC, encompassing either epidermal Langerhans cells (in skin or mucosa), cutaneous dermal or lymphatic follicular dendritic cells (Kashem et al. 2017), potentially influencing the outcome of tolerance induction. Potential routes of AIT comprise subcutaneous and sublingual allergen transfer, both of which are well-established and routinely used. In addition, allergen can be administered via oral, epicutaneous, intradermal, nasal, and intralymphatic routes, which have been and still are the focus of intense investigation for their therapeutic potential (Fig. 1). In the following, we will highlight and discuss these

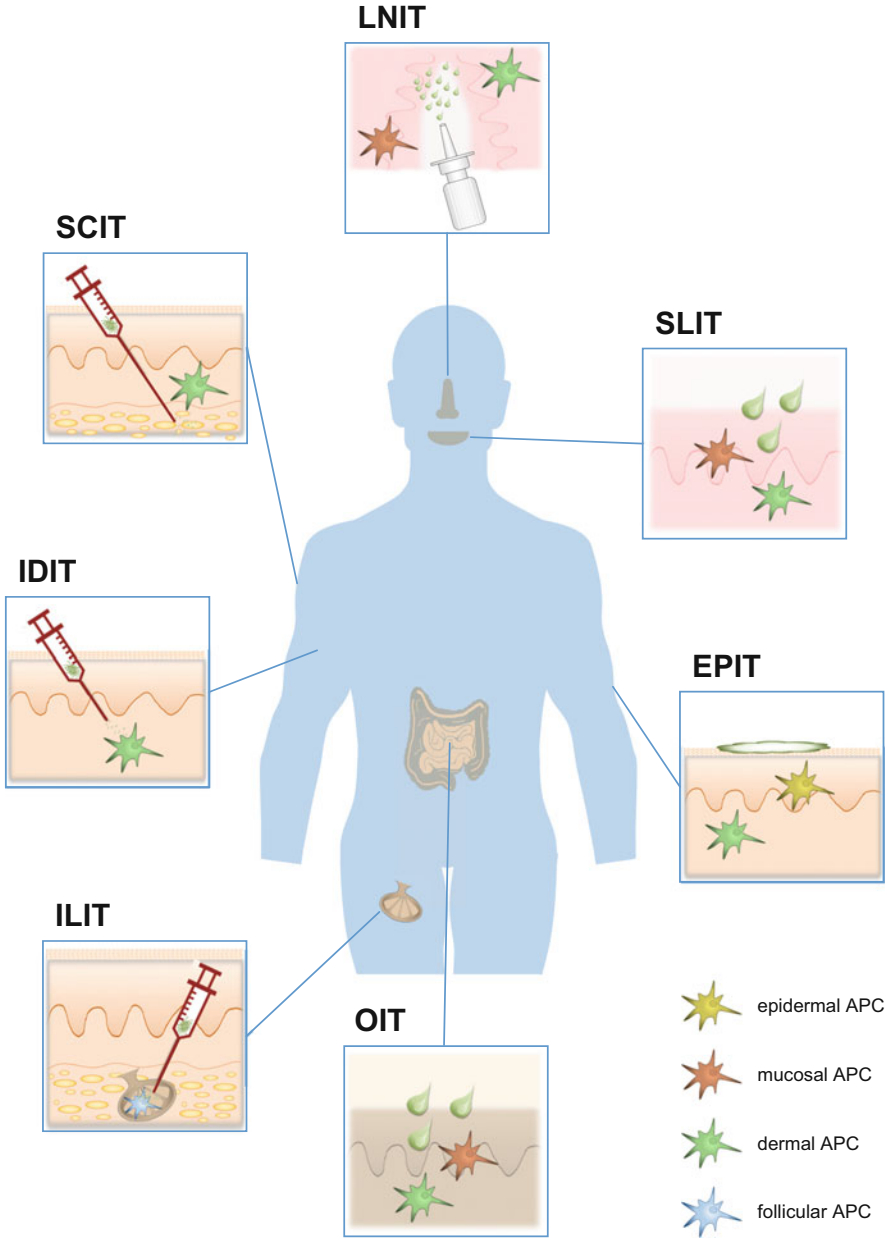


Fig. 1 Different routes of allergen immunotherapy. *APC* antigen-presenting cells

novel approaches, several of which may serve as promising alternatives for future AIT applications in routine practice.

Due to its easy accessibility, the skin constituted the primary venue for delivery of foreign antigens to elicit an immune response, with Edward Jenner performing the first successful vaccination in 1793 by scratching cowpox virus into the skin. Subsequently, the outer epithelium encompassing its deeper layers have been explored and utilized in various ways for the therapeutic application of antigens, not only to establish a protective immune response against infectious pathogens but later on also to induce tolerance against allergens. Here, in 1911 Leonard Noon described the treatment of patients suffering from hay fever by subcutaneous inoculation of increasing doses of grass pollen extract (Noon 1911). Inspired by his and John Freeman's work, who in 1930 published the first rush protocol of grass pollen AIT (Freeman 1930), injection of allergens into the subcutis became the conventional form of AIT, also termed subcutaneous immunotherapy (SCIT). Nevertheless, very soon after Noon's pivotal report, other routes of allergen application have been explored aiming

- to optimize the efficiency of allergen uptake and presentation to the immune system,
- to minimize the risk of systemic side effects by inadvertent vascular delivery, and,
- to establish a convenient, less cumbersome way of delivering the allergen, preferably by self-administration.

For example, as an alternative method topical application of allergens on scarified skin was reported showing clinical efficacy in patients with pollen or animal dander allergy, respectively (Vallery-Radot and Hangenau 1921; Blamoutier et al. 1959). Another, more gentle approach was studied by application of the allergen onto slightly rubbed skin (Pautrizel et al. 1957). In 1986, sublingual administration (SLIT) was proven to be an effective, non-invasive and safe treatment route of house dust mite allergy (Scadding and Brostoff 1986). While initially viewed with skepticism whether SLIT might be as efficacious as SCIT, by now it has been established as an appropriate alternative of allergen delivery in patients suffering from IgE-mediated respiratory allergies like allergic rhinoconjunctivitis and bronchial asthma (Muraro et al. 2018).

As research efforts continued to investigate further routes of allergen application, both clinical studies and animal models have been performed to improve the efficacy and/or safety of AIT. This is exemplified by new forms of treatment including oral immunotherapy (OIT), epicutaneous immunotherapy (EPIT), intradermal immunotherapy (IDIT), local nasal immunotherapy (LNIT), and intralymphatic immunotherapy (ILIT), which we will present here in more detail.

1 Oral Immunotherapy (OIT)

OIT aims to convey protection against anaphylactic reactions to food allergens by inducing allergen tolerance through controlled ingestion of incremental doses of the respective aliment (Wood 2017; Freeland et al. 2017). First reported in 1908 by successful treatment of an egg-allergic child (Shofield 1908), numerous observational and controlled studies of different OIT approaches, both in adults and children, have been presented since then with a broad variety of currently performed clinical trials (Tordesillas et al. 2017a). In general, OIT comprises three phases starting with an initial 1–2 day (rush OIT) dose escalation step to achieve a minimal dose, which is expected to be safely consumed without an increased risk of systemic reactions (Scurlock 2018). This is followed by a build-up phase of several months increasing the amount of applied allergen every (other) week until the target dose (usually between 300 and 4,000 mg of food protein) is reached. Thereafter, the maintenance phase continues, which entails the steady consumption of the attained allergen dose for months to years. The objective is to not only build up a state of desensitization, defined as a transient tolerability of the aliment which has to be constantly renewed by regular allergen intake. Rather, the ultimate goal is to achieve sustained unresponsiveness meaning a long-term allergen tolerance which lasts even when OIT is discontinued.

Most of the studies investigating the immunological or clinical effects of OIT have been performed with cow's milk, hen's egg, and peanuts. Both animal and pre-clinical trials have demonstrated pronounced immune alterations associated with the development of food tolerance, namely a loss of allergen-specific Th2 cells, presumably as a result of either Th2 cell anergy or deletion (Tordesillas and Berin 2018; Ryan et al. 2016). While Treg cells are seen as a central player in mediating allergen tolerance, their significance in OIT is controversially discussed (Tordesillas et al. 2017a). Alterations in different subsets, for example Foxp3⁺ TGF- β - or IL-10-secreting Treg cells, have been observed in OIT-treated patients (Syed et al. 2014; Smaldini et al. 2015), however, others have failed to certify their appearance (Bedoret et al. 2012; Ryan et al. 2016). This might be due to a more local activity at the intestinal side of allergen contact and/or a generally rather transitional role of Treg cells in promoting AIT-specific immunoregulatory processes (Chinthrajah et al. 2016; Möbs et al. 2012). Furthermore, OIT-induced Treg cells may be established on a Th2 cell-like phenotype being rather instable and prone to lose their regulatory capability, which also might explain why food tolerance quite often vanishes after stopping treatment (Noval Rivas et al. 2015). Humoral immune mechanisms have been reported as a delayed decline of allergen-specific IgE (both in peripheral blood and on the surface of effector cells like basophils) and the development of memory B cells producing IgG (namely IgG4) and IgA antibodies, which steadily increase during treatment and can compete with IgE for allergen binding (Tordesillas et al. 2017a; Chinthrajah et al. 2016). It has been shown that OIT-induced IgG antibodies display a high rate of somatic mutations suggesting enhanced allergen affinity compared to IgE and that this antibody subclass can block basophil and mast cell

activation by binding to the inhibitory Fc γ RII receptor (Hoh et al. 2016; Burton et al. 2014).

From the beginning of this century, a variety of clinical OIT studies have been performed mainly with egg, milk, or peanuts, but a few of them also with other foods like wheat, tree nuts, or fish, and most of these studies have been conducted in children and adolescents (Wood 2017; Scurlock 2018). Randomized, placebo-controlled trials have shown that desensitization can be achieved in about 70 to up to 90% of the participants (with a range of 30–100%), reaching tolerated doses of around 1–4 g. However, many will lose their tolerance after stopping OIT with only around 30–40% showing sustained unresponsiveness. Nevertheless, there have been studies with even 90% still being protected when off OIT, as has been demonstrated in very small children of 9–36 months of age successfully treated with peanuts (Vickery et al. 2017). Thus, the management of adverse reactions is still a major challenge in OIT. About 50% of the patients are affected and drop-out rates of 10–20% (in some studies even up to one third of the participants) are very common (Wood 2017, Scurlock 2018). Most of them represent local events like abdominal pain, but systemic reactions are also not uncommon. While the majority of symptoms are rather moderate including urticaria, wheezing, and vomiting, severe anaphylaxis may be possible. Predisposing risk factors are asthma and allergic rhinitis as well as concurrent co-factors like illness, physical stress, or menses. However, for patients with a high risk of food anaphylaxis, OIT represents a very promising therapy to achieve tolerance at least against inadvertent ingestion of the eliciting allergen. Notably, in 2020 the first OIT product has been approved by the Food and Drug Administration in the USA for treatment of peanut allergy in 4–17 years old patients (<https://www.fda.gov/news-events/press-announcements/fda-approves-first-drug-treatment-peanut-allergy-children>; Table 1).

2 Epicutaneous Immunotherapy (EPIT)

EPIT describes the induction of allergen tolerance by topical application of allergens on intact or pre-treated skin (Esposito et al. 2018; Bird et al. 2018). Early reports about its employment date back up to 100 years ago administering the allergen onto either (non-bleeding) scarified or simply rubbed skin (Vallery-Radot and Hangenau 1921; Pautrizel et al. 1957; Blamoutier et al. 1959). Pre-clinical studies in peanut sensitized animals have demonstrated its efficacy by prevention of esophageal inflammation or food anaphylaxis applying the allergen onto intact skin of piglets or mice, respectively (Tordesillas et al. 2017b; Mondoulet et al. 2017). Mouse experiments also shed light onto the responsible immune mechanisms showing a delayed allergen uptake over 48 h by epidermal dendritic cells, which migrated to local lymph nodes (Dioszeghy et al. 2011). Both humoral and T-cellular effects have been noticed in peanut-allergic EPIT models encompassing the synthesis of allergen-specific IgG2a antibodies (without effecting IgE levels), a diminished Th2 cell response and the induction of TGF- β -secreting Treg cells. Interestingly, this Treg cell subset expressed homing receptors not only for the skin but also for the intestinal

Table 1 Summarizing overview of different forms of allergen immunotherapy

Therapy	Schedule		Efficacy	Continued tolerance after stopping therapy	Safety		Compliance
	Up-dosing	Duration			Local AE	Systemic AE	
Approved, routinely prescribed							
SCIT	Yes	Several years	High	Yes (majority)	Common (local swelling)	Rarely (depends on allergen)	Relatively good
SLIT	No	Several years	High	Yes (majority)	Common (oral swelling, itching)	No (anecdotal events)	Relatively good
One product approved in 2020							
OIT	Yes	Several years	High	Small fraction ($\approx 30\%$)	Common (abdominal pain)	Frequently	Hampered
Investigational trials (including phase III studies)							
EPIT	No	Several years	Variable ^a	?	Variable (local eczema)	Rarely (depends on allergen)	Relatively good
Investigational trials							
IDIT	No	Weeks to years	Low ^b	?	Common (local swelling)	No	?
ILIT	No	2 months (3 injections)	High ^c	Yes	Seldom	Rarely	Relatively good
Investigational trials (in use in some countries)							
LNIT	No	Repeatedly	High	No	Seldom	No	?

AE adverse events

^aCompared to OIT rather small increases of tolerated food dosage

^bCompared to placebo

^cOnly few trials with mostly low numbers of patients

allergic effector organs enabling them to execute local immunoregulatory functions (Tordesillas et al. 2017b; Dioszeghy et al. 2017). The decisive role of these Treg cells, which display a CTLA-4-dependent immunosuppressive activity, was demonstrated by both abrogation of the protective effect after depletion of CD25⁺ cells and its conveyance to peanut-allergic mice through adoptive cell transfer.

Clinical studies include double-blind, placebo-controlled EPIT trials in patients (both adults and children) suffering either from allergic rhinoconjunctivitis or food allergies (Esposito et al. 2018). In three studies with grass pollen-allergic adults, the skin was pre-treated either by tape stripping or by abrasion of the corneal layer to enhance the epidermal uptake of allergen. Grass pollen extract solved in either petrolatum or glycerin was administered at weekly intervals for 8 or 48 h under a patch, at dosages of 3–30 µg of the major grass pollen allergen Phl p 5 (Senti et al. 2009; Senti et al. 2012b; Senti et al. 2015). Significant changes were observed for allergen-treated individuals by a reduction of clinical symptoms assessed by visual analogue scale during pollen season, but not by nasal provocation test or a decreased consumption of symptomatic medication. Clinical effects were dose-dependent requiring single allergen dosages of 21–30 µg (cumulative dose of 126 to 252 µg). In another trial with children, patches with 11.25 µg of Phl p 5 were applied at weekly intervals (cumulative dose of 135 µg) for 24 h onto intact skin. Here, a significant improvement in rhinorrhea, nasal obstruction, dyspnea, and ocular tearing was noticed in verum-treated subjects. In contrast, ocular itching rather increased in the verum group, while the use of anti-allergic drugs reduced (Agostinis et al. 2010). Whereas in the children's study no local or systemic events were observed, the majority of EPIT-treated adults reported local reactions, mostly eczema at the patch test area (in up to more than 70%). The frequency of local reactions was related to both allergen dosage and duration of application. Systemic events included pruritus, rhinitis, conjunctivitis, and even asthma, most frequent in patients with abraded application sites.

EPIT trials treating food hypersensitivity utilized patches containing dry powder of allergen, which were placed onto intact skin. Serving as a condensation chamber, the epidermal water loss was herein captured leading to allergen solubilization and hydration of the stratum corneum, thereby promoting epidermal penetration without mechanical skin disruption. While a study in a small number of children treated for a short time of 3 months with single allergen dosages of 1 mg skimmed cow's milk (cumulative dose of 36 mg) failed to demonstrate a significant effect compared to placebo (Dupont et al. 2010), a larger trial in peanut-allergic patients aged 6–55 years showed an increase in tolerated peanut protein [from ≤300 mg (corresponding to roughly one peanut kernel) to up to ≥1,000 mg or 10 times baseline amount, verified by oral food challenge] in about 50% of patients treated with 250 µg of peanut protein daily over 52 weeks (Sampson et al. 2017). The highest response rate was noticed in children <12 years old (53.6% compared to 19.4% in the placebo group). Peanut-specific IgG4 antibodies increased fivefold. A second study in 4–25 years old subjects yielded similar results, again with the highest success among younger children (Jones et al. 2017). In general, treatment with EPIT was tolerated well, with local skin reactions occurring as the most

frequent side effect and absence of systemic adverse events. However, patients with severe peanut anaphylaxis were excluded from participation in these studies. For comparison, a recent published phase III trial of OIT in patients with severe peanut allergy reacting already at doses of ≤ 100 mg peanut protein yielded protection in 67% of individuals tolerating at least 600 mg protein. Notably, protection was only realized in patients between 4 and 17 years, but not in adults (Palisade Group of Clinical Investigators 2018).

3 Intradermal Immunotherapy (IDIT)

Already in 1926, successful treatment of allergic rhinitis was reported by intradermal grass pollen injection (Philipps 1926). Based on this observation and the finding that low-dose intradermal allergen inoculation – as it is known from conventional SCIT – suppresses allergen-induced cutaneous late-phase responses (Rotiroti et al. 2012), a double-blind, randomized phase II trial of IDIT with grass pollen extract was conducted. However, seven pre-seasonal injections of extract containing 7 ng of the major grass pollen allergen Phl p 5 did neither result in improvement of clinical symptoms nor in reduction of symptomatic medication (Slovick et al. 2017). Instead a Th2-priming effect was noticed associated with a trend for worsening of nasal and asthmatic symptoms compared to placebo-treated patients. Another IDIT study treating cat-allergic subjects with peptides encompassing different T cell epitopes of the major cat allergen Fel d 1 at first yielded promising results. Here, the authors could show a significant reduction of nasal and ocular symptoms evaluated by a cat allergen challenge in an environmental exposure chamber (Patel et al. 2013). However, a clinical phase III trial failed to demonstrate efficacy due to an equally high response rate in the placebo group of nearly 60% reduction of the combined total rhinoconjunctivitis score (https://www.circassia.com/wp/wp-content/uploads/2016/06/CIR-PR-17_CATALYST-results-final.pdf). Similar results were obtained by IDIT with peptides of the major house dust mite allergen Der p 1 (https://www.circassia.com/wp/wp-content/uploads/2017/04/CIR-PR-20_HDM-SPIRE-results-final.pdf). Even though different reasons have to be considered for these failures, they have essentially terminated the current interest in IDIT. Besides, intradermal allergen application carries the unpleasant side effect of (sometimes very painful) large local swellings, up to 10 cm diameter, which – although substantiating the immunogenic potential of IDIT – hampers treatment adherence of the patients (Slovick et al. 2017).

4 Intralymphatic Immunotherapy (ILIT)

ILIT describes a very recently proposed concept of allergen delivery transferring the allergen directly into lymphatic tissue by injection into a lymph node, instead of choosing a peripheral route. Increased efficacy in initiating an immunoregulatory response is assumed due to allergen uptake and presentation by follicular dendritic

cells in an immunogenic environment densely populated by T and B lymphocytes (Senti et al. 2011). Thereby, the encounter of allergen-specific T and B cells is highly probable. Methodically, this is achieved by ultrasound-guided administration of the allergen extract into an inguinal lymph node, usually for a total of three injections in intervals of 4 weeks (thus requiring only 2 months of treatment). Human and animal studies mostly have demonstrated immune responses similar to what is known from conventional immunotherapy. Especially the induction of (Foxp3⁺) IL-10-secreting T cells associated with increased production or affinity maturation of allergen-specific IgG4 antibodies, which at least partly were able to block allergen binding by IgE, has been observed in relation to this route of allergen administration (Senti et al. 2012a; Freiberger et al. 2016; Hylander et al. 2016; Kim et al. 2017).

From 2008 until 2019, eight clinical ILIT trials have been performed, either as placebo-controlled or open studies in patients suffering from allergic rhinoconjunctivitis, with the majority conducted in subjects with grass or birch pollen, but also house dust mite, cat, or dog allergy (Kim et al. 2017; Senti et al. 2019). Except for one trial, all proved to be clinically effective. One of them even showed a long-term benefit for up to 3 years analyzing 58 ILIT-treated individuals (Senti et al. 2008). However, most of these studies were executed in small numbers (between 7 and 21 patients) assessing a wide variety of evaluation methods, like different forms of symptom or use-of-medication scores, quality-of-life questionnaires, and nasal provocation tests. One study utilizing a combined score of symptoms and rescue medication – considered as gold standard of testing – failed to show clinical improvement in grass pollen-allergic patients (Witten et al. 2013). It has been proposed that shortening of the injection intervals down to 2 weeks might have been the reason compromising the formation and affinity maturation of IgG-producing allergen-specific memory B cells (Senti et al. 2019), although profound immunological changes like synthesis of allergen-specific IgG4 (as well as IgE) and secretion of IL-10 (as well as IL-4) were noticed (Witten et al. 2013). In terms of safety aspects, injections were mostly tolerated very well, both locally and systemically, even though anaphylactic reactions are possible (Kim et al. 2017).

5 Local Intranasal Immunotherapy (LNIT)

In contrast to the former AIT routes, which aim at treating distant organ or systemic allergic reactions, LNIT focusses on establishing allergen tolerance on the side of allergen application, i.e. the nose as the effector organ in allergic rhinitis (Passalacqua and Canonica 2006). Indeed, clinical benefit is limited to improvement of local symptoms. Introduced in 1951, clinical efficacy of LNIT has been demonstrated in multiple studies conducted mostly in adults since the seventies of the last century (Passalacqua and Canonica 2006; Ascione et al. 2003). Allergens comprised mainly out of various pollen extracts, initially applied as aqueous solutions, which proved to be effective but also resulted in inconvenient local adverse reactions. Utilization of dry powder pump sprays consisting out of granules of 40–50 µm diameter, which were homogeneously deposited at the nasal mucosa,

led to substantially better tolerability. Notably, systemic side effects are virtuously absent underlining the solely stationary impact (Passalacqua et al. 2003). This is further underscored by immunological data revealing local but not systemic production of allergen-specific IgG and IgA antibodies, in conjunction with a decreased allergen-specific T cell response (Giannarini and Maggi 1998; Piazza and Bizzaro 1993). However, obviously, this also implies that allergic unresponsiveness is rather short lasting and dependent on continuous allergen application (Passalacqua and Canonica 2006, Ascione et al. 2003). Thus, LNIT has to be constantly re-applied (either pre-seasonally or persistently) to ensure a long-term clinical benefit in patients suffering from either seasonal or perennial allergic rhinitis.

6 Conclusion

Over the last decades new avenues of allergen transfer, in addition to the well-established methods of subcutaneous injection and sublingual application, namely SCIT and SLIT, have been thoroughly investigated exploring their therapeutic potential, immunological mechanisms, clinical efficacy, and safety aspects (Table 1). Among these, OIT has recently been approved for treatment of peanut allergy in children and adolescents underlining the particular potential of this therapy in the treatment of food allergy, which could not be addressed by the so far established methods of AIT. Clinical studies have shown high efficacy of food tolerance induction, however, current limitations are an early loss of tolerance in many patients when off therapy and a high rate of adverse reactions, mostly in the form of abdominal pain. In contrast, EPIT, which has been also investigated for the treatment of food hypersensitivity (in addition to allergic rhinoconjunctivitis), is characterized by a good safety profile and the ease of application. However, therapeutic effects have been modest so far requiring further improvement in providing a topical allergen delivery system, which releases enhanced amounts of allergen into the skin while causing little or no irritation. Future applications might especially target individuals suffering from severe anaphylaxis with a high risk for strong adverse reactions at even low-level allergen exposure, with children probably showing the best response. Another interesting approach is ILIT, which has shown promising results in treating allergic rhinoconjunctivitis, albeit mostly in small numbers of patients. A significant advantage seems to be the low number of injections needed to achieve prolonged allergen tolerance, possibly through direct targeting of the immunoregulatory lymphatic tissue, with a reasonable safety profile. However, this requires appropriate technical experience in ultrasound-guided allergen administration into the (inguinal) lymph nodes. In comparison, LNIT can be easily performed by the patient him- or herself for the treatment of allergic rhinitis without obvious risks for pronounced adverse events. However, unlike SLIT as a routinely used form of mucosal AIT, it shows only temporary benefit necessitating its long-term, regular administration.

In summary, there are several interesting alternative routes of allergen administration, which are characterized by distinct features in regard of their immunological

action, responsive allergic diseases or individuals, and potential side effects. Here, refinements are needed to target current limitations in terms of therapeutic efficacy and/or clinical safety. This also requires the conduction of well-designed multi-center randomized, double-blind, placebo-controlled studies in large number of patients, in particular addressing questions of optimal allergen dosages and composition, treatment schedules, targeted groups, of how to achieve long-term tolerance and of biomarkers indicating potential therapeutic responses and to expected unwanted side effects.

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