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REVIEW ARTICLE

Review Series: Immunotherapy and Tolerance—Cutting Edge

Sublingual Immunotherapy: Recent Advances

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ABSTRACT

The practice of administering sublingual immunotherapy for respiratory allergy is gaining more and more diffusion worldwide as a consequence of the robust demonstration of clinical efficacy and safety provided by recent high-powered and well-designed studies, confirming for individual seasonal allergens the results of previous metanalyses in adult and pediatric populations. Preliminary evidence derives from recent rigorous trials on perennial allergens, like house dust mites, and specifically designed studies addressed the benefits on asthma. Emerging research suggests that SLIT may have a future role in other allergic conditions such as atopic dermatitis, food, latex and venom allergy. Efforts to develop a safer and more effective SLIT for inhalant allergens have led to the development of allergoids, recombinant allergens and formulations with adjuvants and substances targeting antigens to dendritic cells that possess a crucial role in initiating immune responses. The high degree of variation in the evaluation of clinical effects and immunological changes requires further studies to identify the candidate patients to SLIT and biomarkers of short and long term efficacy. Appropriate management strategies are urgently needed to overcome the barriers to SLIT compliance.

KEY WORDS

allergy, desensitization, recent advances, SLIT, sublingual allergen immunotherapy

INTRODUCTION

The traditional subcutaneous route of administration for allergen immunotherapy (SCIT) repeatedly demonstrated to be effective in respiratory allergy. Nonetheless, with injections some risk of severe or even fatal adverse events still remains, partly attributed to technical or human errors.[1,2] Since a large part of the reactions appear unpredictable despite that all precautions are taken, alternative routes of administration were sequentially explored. The sublingual route for administering allergen immunotherapy (SLIT) was introduced for the first time in 1986.[3] Despite initial skepticism owing to the missing characterization of the extracts used and the poor design of the early studies, SLIT gradually revealed itself as a promising convenient route.[4] Numerous randomized controlled trials confirmed its clinical efficacy and post-marketing surveys supported the good safety profile during the last 15 years. Owing to these clinical evidences, SLIT is officially accepted in international consensus documents as a viable alternative to SCIT for both adults and children[4-6] and it is currently prescribed at least as frequently as SCIT, representing in some European countries 80% or more of new immunotherapy prescriptions.

Current research on SLIT is focused on confirming the efficacy for all the different relevant allergens (grass, birch, ragweed, house dust mites, cat), on a better definition of allergen extracts and the improvement of their safety and the immunological properties, on the identification of best treatment regimens, on the possibility of extending the clinical indications. In this review we describe the most recent step forwards in these fields of the SLIT development (Fig. 1).

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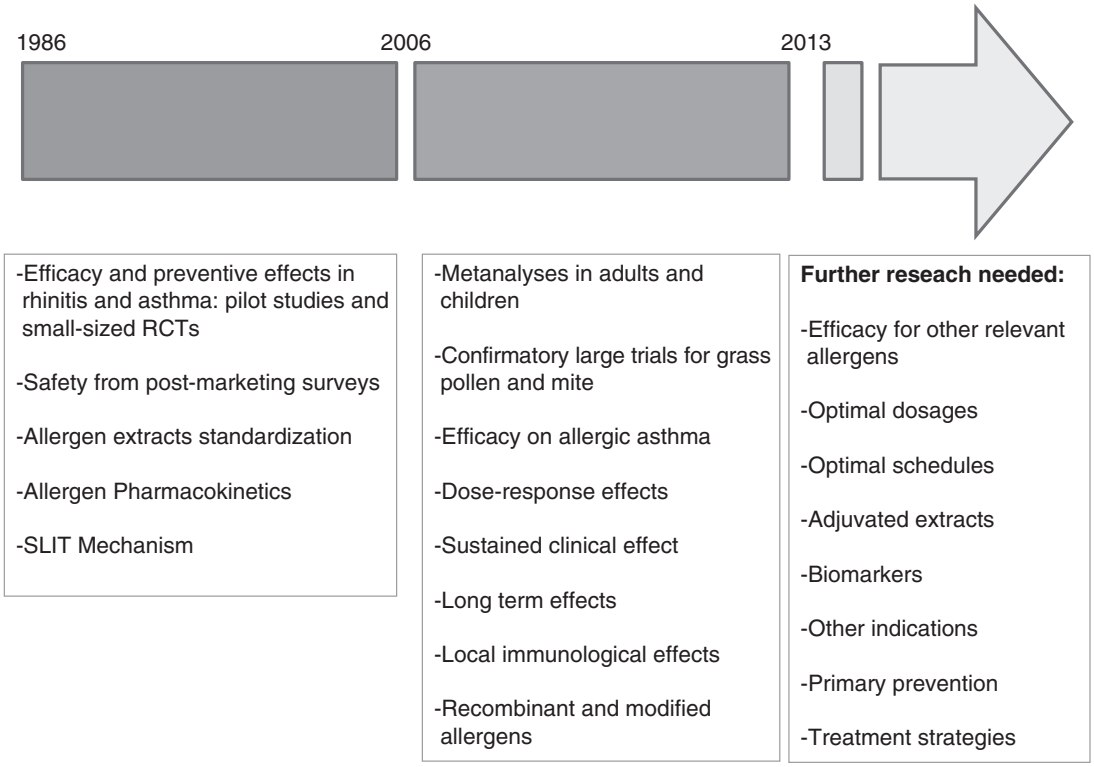

Fig. 1 The main steps in SLIT scientific advance.

THE CLINICAL EFFICACY OF SLIT

To date, the principal indication to SLIT remains allergic rhinoconjunctivitis and most of the clinical data derive from trials conducted in patients with that disease mainly in European countries. In more than 60 positive studies, two-thirds of them in dust mites and grass allergy, the degree of clinical effect ranged from 10 to 45% over placebo with greater than 20% in about two-thirds.[6]

Meta-analyses suggested the efficacy of SLIT in allergic rhinitis and asthma, in adults and children,[7-10] although some concerns were raised about the risk of publication bias, high heterogeneity and shortcomings of clinical trials.[11,12] The benefit was clearly demonstrated in successive phase II and phase III trials with grass tablets, in which a dose-dependent gradient was apparent and the magnitude of effect, sustained along the treatment, ranged from 25 to 50% over placebo.[6] Preliminary reports from a very recent randomized, multicentre, double-blind, high-powered study on the efficacy and safety of mites tablets showed that symptoms of allergic rhinitis were reduced compared to placebo after one year of treatment.[13]

Very few small randomized trials compared head-to-head SLIT with SCIT and could not demonstrate a difference between the two routes. Indirect comparisons by metanalysis did not provide conclusive results,[14] although for grass pollen a more prominent effect was in favor of SCIT.[15]

SLIT efficacy and safety have been well established in several US clinical trials utilizing single allergen tablets or glycerinated extracts of grass pollen, ragweed and dust mite.[16] Although SLIT does not have regulatory approval in the United States, but it is used in clinical practice, recent systematic reviews describing the effectiveness and safety of SLIT (off-label use of subcutaneous-aqueous allergens for sublingual desensitization) compared with other therapies for the treatment of allergic rhinoconjunctivitis and asthma, concluded that the body of evidence provides a moderate to high support, but high-quality studies are still needed to define optimal dosages.[17,18] No life-threatening adverse events were described, although limitations in the standardization of adverse events reporting were noted. This issue has been recently faced by the scientific community, since there is no universally accepted system to grade and classify SLIT adverse events. A World Allergy Organization Taskforce proposed a clinically based grading of the severity of local adverse events, that are rather common with SLIT, based on the Medical Dictionary for Regulatory Activities nomenclature system in order to improve and harmonize surveillance and reporting of the safety of SLIT.[19]

Some SLIT studies also evaluated the effects in asthma, although rarely asthma was the primary out-

come.[6] As a consequence none of them has been adequately powered or specifically designed to avoid biases. Only recently a positive therapeutic effect on asthma control was demonstrated by a reduction of more than 80 μg/day inhaled budesonide for the SLIT group compared to placebo after 1 year of daily treatment, in a trial investigating whether the treatment of 602 asthmatic patients allergic to house dust mites with sublingual tablet can reduce the need of inhaled corticosteroids.[20] An ongoing study will be able to establish whether this treatment can reduce the frequency and the time to first exacerbation after inhaled steroids reduction.[21] The potential steroid sparing effect, that acquires particular relevance considering the double exposition (nasal and bronchial) in patients with AR and asthma, has been suggested also by another recent study investigated whether SLIT with chemically modified allergen extract, provides any advantage in real-life conditions and in a relatively long term period, in achieving the control of seasonal mild persistent asthma related to birch pollen.[22] The high treatment tolerability in these adult patients non adequately responding to fixed low-dose of inhaled budesonide, suggests the perspective that vaccines with reduced allergenicity could overcome the limitations due to safety issue in uncontrolled or severe asthma.

EXTENDING THE CLINICAL INDICATIONS OF SLIT

Due to the favorable safety profile and acceptance by patients, the use of SLIT has also recently been proposed in non-respiratory allergy, including atopic dermatitis (AD) and food allergy as explorative areas of application.[23]

A number of observational studies suggested that specific allergen immunotherapy may be a promising treatment for AD, particularly when a IgE-mediated component of the disease is involved.[24] Atopic eczema is a multifactorial disease, including complex genetic modifications, responsible for skin barrier impairment, and combinations of environmental and endogenous factors that can direct its course. However there is agreement on the possible link between some forms of AD and allergic sensitization, mainly to house-dust mites and foods, although some concerns derive from the selection of the most relevant allergen for desensitization, because patients are frequently polysensitized.[25] In a double blind randomized controlled trials, SLIT with house dust mites extract was given to 5-16 years children with AD stratified according to disease severity for 18 months in addition to rescue therapy.[26] With respect to controls, from the 9th month onwards, patients with mild-moderate disease allocated to active group achieved a significant improvement in the SCORing Atopic Dermatitis [SCORAD] and use of medications. Very recently SLIT mite drops, given to 58 Asiatic patients randomly compared to controls receiving only pharmacotherapy, appeared safe and effective in a 12 months follow-up and could induce a tolerogenic IgG4 response to mite allergen correlated with the favorable clinical efficacy.[27] More controlled studies involving a sufficient and representative numbers of subjects are required to define the value of SLIT in AD. Phase II trials are currently ongoing[28] and new experimental approaches contemplate the use of SLIT in AD to investigate the primary prevention of allergic sensitization and respiratory allergy. This "early intervention" aimed at arresting the process before it becomes persistent, was the strategy of The Global Prevention of Asthma in Children (GPAC) Study, a double blind placebo controlled trial to test the hypothesis that enhancing the levels of mucosal exposure of children at high risk of inhalant allergy prior to the onset of sensitization would reduce the likelihood of subsequent sensitization and/or development of asthma.[29] Unfortunately firm conclusions could not be drawn by this small pilot study, likely because of the objective difficulties in making infants retain the sublingual drops under the tongue for enough time to maximize mucosal penetration of the appropriate dose required for triggering immunologic processes.

Conditions like IgE-mediated food allergy, for which dietary avoidance represents the common approach, may be good candidate for immunotherapy. More than 10 years ago, preliminary studies investigating SCIT in peanut allergy, showed uncertain result for benefits and unacceptable high risk for systemic reactions, prompting research to explore the potential of different administration routes.[30,31] Concerning SLIT, encouraging results came in the last decade from five randomized controlled trials. In 2005 Enrique and colleagues observed a significant increase in the symptoms threshold to oral food challenge with hazelnut in 12 adult patients receiving SLIT, with a rate of systemic reactions equal to 0.2% of administered doses.[32] Similar results were found in peach-allergic patients, treated with a purified extract standardized for Pru p 3 content (lipid transfer protein of peach), along with a reduction of the skin specific reactivity. Numerous adverse events occurred, mainly mild and self-resolving, but 16 reactions were systemic.[33] In 2011 peanut-allergic children following 6 months of dose escalation and 6 months of maintenance dosing were able to tolerate 2500 mg (20 times more peanut protein than the placebo group) during the oral challenge, showing reduced skin response to prick test and an curious increase of salivary specific IgA-levels.[34,35] Dosing side effects were primarily oropharyngeal and uncommonly required treatment. The clinical outcomes in all these three studies were accompanied by the increase of IgG4 levels, but inconsistent variations in IgE levels, IL10 and other markers of immune-regulation. The most recent mul-

ticenter study for peanut SLIT involved forty subjects and after 44 weeks of therapy 70% of SLIT treated patients developed partial desensitization, compared to only 15% of the placebo group.[36] Oral immunotherapy seems to be the most promising approach based on the results from recent abundant literature.[37] A randomized study found that SLIT was less efficacious for cow milk allergy desensitization than oral immunotherapy, but was accompanied by fewer systemic side effects.[38] These findings were confirmed by a recent retrospective study comparing peanut-allergic individuals treated with either oral immunotherapy or SLIT.[39] Finally in the last three years an interesting approach consisted in the use of SLIT with inhalant allergens to treat oral allergic syndrome induced by cross-reacting foods.[40-42] Several immunological changes have been related to the effects of immunotherapy in food allergy, but whether immunotherapy is able to induce only desensitization, where continuous allergen exposure increases the threshold of clinical reactivity to the food, or tolerance, that is the ability to consume a food without allergic symptoms after treatment is ceased, is still matter of research.

Latex allergy seems to be a promising field of application for SLIT but owing to the partial discrepancy of the results of the available studies, it has not yet been accepted as an indication to SLIT, although standardized extracts are commercially available and used.[43]

Finally the potential of SLIT for hymenoptera venom allergy was investigated in a couple of proof of concept studies. In the first experience, 21 subjects with history of systemic reactions to wasp sting, were safely treated with sublingual vespula extract. No significant immunological changes were found during the treatment, but when 4 patients were stung again, just 1 experienced isolated throat constriction.[44] To date only one randomized placebo-controlled study was carried out in this field.[45] This pilot trial administered honeybee venom to 15 subjects with history of large local reactions only. At the sting challenge after six months of treatment, a significant reduction of the wheal size was observed in the active group only, and in 57% of patients the reaction was more than halved. No adverse event was reported, but some concerns derived from the ambiguous immunological changes in respect to what was normally observed with injections. These encouraging explorative findings suggest that dose-finding studies and larger trials are needed to investigate the feasibility of SLIT in patients with hymenoptera venom allergy.

SLIT AND THE NATURAL PROGRESSION OF RESPIRATORY ALLERGY

Immunotherapy for respiratory allergy is considered as an adjunct to the pharmacological plan for the immediate purpose of reducing symptoms and the need for rescue medications.[46] On the other hand immunotherapy acts as a biological response modifier and induces profound changes in the immune response to allergens, able to affect the natural progression of the disease in the long term. This 'preventive effect' has been shown with SLIT in a number of open randomized controlled trials.[47,48] Another important aspect not shared with the standard pharmacological treatments is the long-lasting effect after discontinuation, that has been seen observed in several SLIT studies in adults and children.[49-53] According to the literature, the beneficial effects are maintained for 2-6 years after discontinuation of SLIT, nonetheless, a formal demonstration of this long-lasting effect would require prolonged double-blind controlled trials, which are not feasible from a practical and ethical viewpoints. At present the results of randomized, double-blind, placebo-controlled, multinational, phase III trials, including 2 years of blinded follow-up after completion of a 3-year period of treatment, confirm the disease modification by grass immunotherapy tablet, but further studies are needed to address the potential long-term effects for other seasonal and perennial allergens and to identify potential biomarkers of tolerance.[54,55]

All these 'preventive' effects gain particular interest when considering the cost-effectiveness of the treatment. A recent health technology assessment suggests that SLIT when compared with pharmacotherapy may become convenient from around 6 years, but more robust estimates are needed to reach definitive conclusions.[56]

IMPROVING THE SAFETY OF SLIT

The incidence of local side effects with SLIT (notably mild itching and swelling of the lips and floor of the mouth) has been estimated on average about 35%, but typically it is observed up to 85% of patients in clinical trials[57]; these events usually appears within minutes or hours after intake and are of short duration (less than 14 days), frequently self-resolving or requiring dose adjustment. Systemic reactions such as urticaria, angioaedema and asthma, although seldom, may occurs more frequently during dose escalation. Albeit a dose-response relationship for safety is not formally defined with SLIT, in part because of the lack of a universal grading system, the occurrence of side effects may be dose-dependent and allergen-dependent.[58,59] No fatal events has ever been described but literature quotes twelve cases of anaphylaxis to SLIT, associated to multiple pollen allergen, rash induction with latex extract, over-dosage and high-dose SLIT in patients with previous reactions to injective SIT.[60,61]

The efforts to improve the intrinsic safety of allergen extracts represent an interesting approach for the treatment optimization. Recombinant hypoallergenic allergens and allergoids have been precisely developed with the aim of reducing the risks for therapy-associated side effects. Recombinant DNA

technology fully guarantees the characterization in terms of physical, chemical, and immunologic properties in absence of non-allergenic proteins, polysaccharides and contaminants in respect to extracts of natural source materials. This promising approach, leading in the future to the 'patient's tailored immunotherapy', can take the advantage of hypoallergenic variants specifically developed with the purpose of increasing the administered doses and simultaneously reducing the IgE-reactivity and the consequent risks for therapy-associated side effect.[62] Hypoallergenic variants are well suited for subcutaneous application, whereas it has been argued that wild-type recombinant allergens are preferred for sublingual application, due to the expected relevance of IgE-facilitated allergen presentation by oral Langerhans cells in the promotion of a regulatory T-cell response. Recombinant allergen products for SLIT are in development, and one of the first of these is based on tablet formulations of rBet v 1,[63] but due to regulatory and marketing problems, the use of recombinant allergens seems likely in the more distant future

The chemical modification of native allergens in order to reduce their IgE-binding activity, as shown by in vitro (immune-inhibition assays, basophil activation, and basophil mediator release) and in vivo techniques (skin testing and nasal provocation), produces hypoallergenic preparations that retain the T-cell reactivity (antigenicity), and the ability to induce allergen-specific IgG antibody response (immunogenicity), both essential for the clinical effects.[64] However the chemical modification traditionally obtained by reaction with glutaraldehyde or formaldehyde, produces polymeric allergoids, with high molecular weight, suitable for injective route only.[65-67] The carbamylated allergoids, obtained with potassium cianate by an extremely selective substitution of the extract lysine residues, maintain structural conformation and low molecular size (monomeric allergoids) necessary for mucosal absorption. Preparations based on carbamylated allergoids currently represent the sole chemically modified allergens suited for sublingual administration[68,69] and numerous post-marketing studies documented the optimal safety profile, with incidence of side effects lower than 10% of treated patients.[70-72]

IMPROVING THE IMMUNOLOGICAL PROPERTIES OF SLIT

SLIT has been shown to work differently from SCIT, being the extract captured by dendritic cells in the oral mucosa (expressing high levels of FcεRI receptors, MHC class I and II and costimulatory molecules compared with their skin counterparts) and migrated to draining lymph nodes, where regulatory or suppressive T cells secreting IFN-γ and/or IL-10 are stimulated and blocking IgG1 and IgG4 antibodies are generated. Some SLIT preparations have been developed with the aim of amplifying the effect by modulating the immune response to the therapy.

One of these strategies contemplates the use of adjuvants (either bacterial or DNA-based), substances with the potential of enhancing the immunogenicity of antigens or allergens and largely investigated for injective immunotherapy. Oral dendritic cells may be the ideal target cells for adjuvanted SLIT vaccines, enhancing the tolerance mediated by these cells mimicking the natural contact of the individuals' immune system to allergens.[73] Recently probiotics as adjuvants for SLIT have been investigated in mice models resulting in a enhanced tolerance induction.[74,75] Another approach investigated the use of detoxified bacterial toxins or carbohydrate polymers adjuvanted to allergens as 'microparticles' and mucoadhesive particles, substances that could improve the contact of allergenic extracts with the oral mucosa.[76-79] The research field dedicated to the development of the molecular structure of SLIT extract to optimize the interactions with antigen presenting cells seems particularly attractive and primising.[80] Encouraging preliminary clinical findings in humans derive from the first phase I/II, dose-ranging, placebo-controlled trial with MPL-adjuvanted SLIT.[81]

Chemically modified allergen preparations suitable for sublingual administration, obtained with carbamylation of the native allergen in order to maintain its molecular dimension,[82] have been developed and revealed clinical efficacy and immunological effect in several clinical trials.[83,84] Carbamylated allergoids, in addition to a reduced IgE-binding activity, showed enhanced bioavailability in pharmacokinetics studies as a consequence of the partial resistance to enzymatic degradation.[85] This peculiarity is expected to enhance the amount of extract implicated in the tolerance induction, by coupling the stimulation of the oral mucosa-associated and gut-associated immune system with systemic absorption.

In the last decade some trials with dose-ranging design had demonstrated a dose-dependent clinical effect of SLIT with native extracts and identified the optimal maintenance dose for grasses in 15-25 μg major allergen per day (approximately 50-times the monthly dose of SCIT).[86] The amplified features of SLIT preparations using adjuvants and modified allergens suggest that an adjusted dose tuning and dose response effect evaluation is specifically required in respect to SLIT traditional preparations.

ADDRESSING THE UNMET NEEDS

Recent advances in the field of SLIT addressed the unmet needs remarked by the official positions of the scientific community.[6] The identification of the candidate patient, who would receive the most benefit from SLIT, is still a challenge because no predictive biomarker of efficacy has been identified so far, likely because of the difficulties in establishing direct corre-

lations between the variability of clinical endpoints and the variety of immunological and inflammatory changes observed during the treatment.[87] If recent research in SCIT strengthen the value of IgE-FAB inhibition and IgE-blocking factor as biomarkers of the clinical response,[88] for SLIT a molecular changes at the level of dendritic cells, like the expression of Complement component 1 (C1Q) and Stabilin-1 (STAB1), have been suggested as an early event indicative of the subsequent orientation of adaptive immune responses.[89]

Currently no conclusive data exist on which is the best administration regimen (preseasonal, coseasonal, pre-/co-seasonal or continuous) of a SLIT course, since direct head-to-head comparisons are scarce. For pollen allergies a comparable efficacy results from preseasonal an precoaseasonal schemes and these approaches seems to be the best choice also from an economical viewpoint.[90-92] Continuous regimen appeared more effective than coseasonal during the first year, but in the subsequent years both seem equivalent.[93] For what concern the optimal duration of a SLIT course, a study suggests that a 4-year duration is recommended because it induces a long-lasting clinical improvement similar to that seen with a 5-year course and greater than that of a 3-year vaccination, but further research is needed to draw conclusions.[51]

The standardization of allergen extracts is of primary relevance to the clinical efficacy of SLIT, but a certain variability has been described in the biological potency among different extracts of some allergens.[94] Manufacturers, in fact, have developed extensive protocols for standardization and quality control, but each company is using its own in-house reference standards and units to express potencies. Although some products reports the content of major allergen in micrograms, comparisons between these information should be considered with great caution in absence of accepted reference standards for the materials and methods of quantitative analysis. A universal standardization of the extracts would allow better comparison of the various trials and products. The CREATE project provides a model for the development of a comprehensive panel of international reference preparations that will harmonize allergen measurements worldwide.[95]

Despite that we entered the 'molecular era' of allergy, combination vaccines, mimicking natural exposure conditions, are still widely used. They offer a broad coverage to the allergic patients by extending the repertoire of allergens, however the potential risk of de novo sensitization to epitopes present in the vaccine theoretically exists. Some cases of neosensitization have been described with SCIT, nevertheless the risk should be reduced when the allergen is delivered in an immune environment prone to tolerance induction like the oral mucosa, as recently observed for grass pollen and mite SLIT.[96,97]

Finally there is an need for identification of the potential barriers to an optimal compliance to SLIT. A recent retrospective analysis of a community pharmacy database from The Netherlands, including 2796 patients who received SCIT and 3690 who received SLIT, warned that the real-life persistence to the treatment is better in SCIT than in SLIT users, but it is low overall.[96] An adequate action plan, including education, frequent contacts, and strictly scheduled visits recently obtained a significant reduction of SLIT discontinuations,[97] suggesting that the development and implementation of measures that will enhance persistence and compliance to SLIT are urgently needed.

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