

Allergen-specific immunotherapy in asthmatic children: from the basis to clinical applications

Expert Rev. Vaccines 12(6), 639–659 (2013)

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Atopic asthma in childhood with the tendency to persist into adult life is an important issue in pediatrics. Allergen-specific immunotherapy (SIT) is the only curative treatment option for these children, being directed to the causes of the disease. The Th2 phenotype is a predominant immunological pattern in atopic asthma and SIT leads to apoptosis/anergy of T cells and induces immune-regulatory responses and immune deviation towards Th1. Many factors can affect the safety and efficacy of SIT, such as pattern of sensitization, allergy vaccine (allergen extracts, adjuvants and conjugated molecules), route of administration (subcutaneous or sublingual) and different treatment schedules. Overall, asthma symptoms and medication scores usually decrease following a SIT course and the most common observed side effects are restricted to local swelling, erythema and pruritus. Compared with conventional pharmacotherapy, SIT may be more cost effective, providing a benefit after discontinuation and a steroid-sparing effect. In addition, it can prevent new sensitizations in monosensitized asthmatic children. Microbial supplements such as probiotics, immunomodulatory substances like anti-IgE/leukotrienes, antibodies and newer allergen preparations such as recombinant forms have been tested to improve the efficacy and safety of SIT with inconclusive results. In conclusion, SIT provides an appropriate solution for childhood asthma that should be employed more often in clinical practice. Further studies are awaited to improve current knowledge regarding the mechanisms behind SIT and determine the most appropriate materials and schedule of immunotherapy for children with asthma.

KEYWORDS: adjuvant • allergen • asthma • immunoglobulin E • immunotherapy • Pollinex® Quattro • probiotics • vaccines

Asthma is one of the leading causes of school absenteeism and its incidence has increased, mainly in the pediatric population, over the course of the last decade [1]. While half of asthmatic adults suffer from atopic disease, this percentage is much higher in the pediatric population, involving two-thirds of patients [2]. Chronic inflammatory processes have been observed behind both allergic and nonallergic asthma [3]. An inappropriate response of the immune system to aeroallergens is responsible for both the initiation and exacerbation of asthma, especially in children with an immature immune system [4]. For this reason, allergen-specific immunotherapy (SIT) represents the only curative option, being directed against the underlying mechanisms of atopic asthma, potentially providing a chance

to modify the natural course of atopic asthma, mainly in afflicted children [2,5]. SIT is defined as the gradual administration of increasing amounts of allergen extracts in allergic subjects in order to induce tolerance and a reduction of allergic symptoms following natural exposure [6].

The introduction of SIT dates back to 1911 with Noon and Freeman's experiences treating allergic rhinitis (AR) [7]. Since then, much progress has been made, not only in the disclosure of the mechanisms underlying allergies and SIT mechanisms, but also in the optimization of SIT procedures in clinical practice. Currently, this treatment is indicated for respiratory allergy and venom allergy [6]. The clinical efficacy of SIT has been largely investigated in rhinoconjunctivitis and asthma [8–10]. Future indications may

include other allergic conditions [11], such as food allergy [12], atopic dermatitis [11] and latex allergy.

Outdoor allergens, including grass, ragweed and tree pollens, and indoor allergens such as house dust mites (HDMs), animal danders and molds, are recognized triggers of allergic symptoms in susceptible children and their extracts have been variably used in SIT in order to induce tolerance [9].

In clinical studies, the treatment protocols of SIT show wide variability, with continuous, preseasonal or coseasonal regimens and duration ranging from only 2 months to 5 years [8–10]. Another variable is the formula of allergy vaccine used, frequently adopting different extract dosages or adjuvants to enhance the immunological effect and reduce the risk of side effects [9].

SIT is generally well tolerated, but local or systemic adverse reactions may occur. The most feared and potentially fatal event is anaphylaxis, which has been observed more frequently among asthmatics following SIT injections [10]. The risk of anaphylaxis has been estimated as one case per 2,500,000 administrations [13]. For this reason, careful supervision by trained clinicians, familiar with subcutaneous immunotherapy (SCIT) procedures and the management of possible systemic reactions, is recommended [8].

Since the selected route of administration could impact the safety of SIT, sublingual immunotherapy (SLIT) gained popularity in recent years due to its good efficacy and safety profile (only 12 cases of anaphylaxis have been documented) [14,15].

Furthermore, SIT has been shown to prevent the occurrence of new sensitizations in monosensitized children [16–19] and to improve patients' quality of life [20], with benefits lasting for several years after cessation of treatment [21,22]. Additionally, it provides protective effects in children with rhinoconjunctivitis from asthma development [23]. Thus, the overall cost-effectiveness of this treatment may be superior compared with conventional pharmacotherapy for childhood asthma [8].

The aim of this article is to review medical literature in order to summarize the evidence of efficacy, the immunological effects and the main aspects able to favorably affect the outcomes of a SIT course in asthmatic children.

Search methodology

Primary search methodology

A systematic search was performed to compare the efficacy and safety of different routes of SIT administration, with respect to outdoor and indoor allergen extracts, in comparison with placebo in asthmatic children. The authors searched MEDLINE and Scopus databases without language restriction for randomized double-blind clinical trials (RCTDB) published from January 2000 to June 2012. Furthermore, some articles were added through the references cited in the retrieved publications. A total of more than 1000 articles were identified with the keywords 'immunotherapy' and 'asthma.' The inclusion criteria were restricted to RCTDB studies that investigated the efficacy of SIT in asthmatic patients, or other allergic conditions only if associated with asthma, in participants younger than 18 years of age (FIGURE 1). Studies that investigated the preventive effect of SIT in asthma development and studies not available in full-text form

were excluded. Primary search results included a total of 29 studies. SLIT was used in 19, SCIT in eight, and two studies employed both of these routes. With respect to SLIT, eight studies were on asthma related to pollens, eight studies were on asthma related to mites and one study was on asthma related to natural rubber latex. Through the 3-month to 3-year treatment, 14 studies reached favorable results, three studies found equivocal benefits and two studies assessed only the safety of SLIT, with promising results. We found eight eligible articles that employed SCIT routes, with five studies in mite asthma, two studies in mold asthma and only one article focused on pollen asthma. Favorable results were defined as improvement in symptom scores or rescue medication and equivocal results were referred to the situation in which no significant improvement was observed. In this way, six out of eight (75%) studies found favorable results from SCIT and only two of them failed to show clinical improvement during SCIT.

Scoring the quality of included studies by primary search methodology

The quality of clinical trials retrieved by primary search methodology was assessed by Jadad criteria as previously published [24]. Briefly, the quality of the trial was quantified using three questions regarding randomization, double blindness and reporting the withdrawals. Scoring ranged from zero to five; TABLE 1 demonstrates Jadad scoring. Scoring of the trials was performed by two of the authors of this review (Z Aryan and N Rezaei).

Secondary search methodology

In order to find studies focused on the mechanisms behind SIT or recent aspects of SIT using pre/co-treatments and novel adjuvants, MEDLINE, Scopus, EMBASE and the Cochrane Database of Systematic Reviews were searched with the keywords 'asthma', 'immunotherapy', 'subcutaneous immunotherapy', 'sublingual immunotherapy', 'adjuvants', 'probiotics' and 'allergy' with no limitation on time. The aim of this secondary search was to provide further information regarding different aspects of SIT that are useful in clinical practice.

The results of the primary and secondary searches have been organized into subsections and are discussed in detail. Results of primary search methodology are outlined in TABLES 2 & 3, stratified with respect to the route of administration and discussed in the section on 'Route of administration'. 'Rationales to alter the course of childhood asthma using SIT' highlights the immunologic basis of SIT. The section on 'Asthma triggers and materials of SIT; two sides of a coin' describes allergens and their extracts known to trigger atopic asthma in children to provide vaccines appropriate for SIT. Adjuvants of allergy vaccines and pre-/co-mediations that have been tested in SIT schedules to improve their safety and efficacy are described in 'Adjuvants and conjugated molecules', 'What is the role of mycobacteria and probiotics in allergen immunotherapy?' and 'Modifications in routine SIT procedure; advantage or disadvantage?'. Moreover, discussion of findings and the authors' viewpoint regarding evolution of the field over the next 5 years are explained in 'Expert commentary' and 'Five-year view', respectively.

Rationales to alter the course of childhood asthma using SIT

Inappropriate response of the immune system to aeroallergens in susceptible children with genetic predisposition is responsible for the initiation of atopic (extrinsic) asthma in early life [25]. In childhood a wide variety of asthma phenotypes are possible, from intermittent viral infection-associated wheeze to atopic asthma, but only the atopic type is most likely to persist into adult life, indicating the value of a curative treatment for this subset of patients [26–28]. In the early phase, allergens are introduced to the immune system by APCs, which induce maturation of different subtypes of T-helper cells from regulatory to Th2 cells [29]. With the predominance of the Th2 phenotype, the result of exposure would be allergic manifestation and inflammation in re-exposure instead of tolerance induction [30]. As depicted in FIGURE 2, costimulatory molecules construct important components of this procedure in which expression of B7 molecules, Fas or even no costimulatory substance leads to T-helper differentiation, apoptosis or anergy, respectively.

Specific IgE binds to mast cells and basophils, enabling them to react with aeroallergens in an inflammatory manner with the production of IL-4, IL-5 and IL-13, which subsequently induce hypersensitivity features and remodeling in the airways [31,32].

The main immunologic mechanism behind allergen immunotherapy is tolerance induction using either SCIT or SLIT [33,34]. CD4⁺ Th cells are pivotal players either in promotion of allergic reactions or tolerance induction by SIT.

Apoptosis, anergy and immune deviation are achieved during SIT (FIGURE 2) [35].

Immune deviation from allergic Th2 responses to Treg induction and/or Th1 activation is the cornerstone of immunotherapy [36]. Following this switching, IL-5, IL-13 and IL-4 production will be decreased and IFN- γ and IL-12 production will be increased [37–39]. Another deviation which is more important is the induction of suppressive Treg responses and IL-10 and TGF- β production [40]. This is clearly demonstrated that in parallel to downregulation of Th2 response, CD4⁺CD25⁺ suppressor cells expressing IL-10 are upregulated during SIT in human and animal models [40–42]. Tregs suppress IL-17 production by Th17 cells and subsequent inflammation [37]. These cytokine changes result in an alteration in antibody profile [43–45] from IgE reduction to

high levels of IgG blocking antibody induction [46]. In humans, IgG4 level increases during SIT (positively correlated with the allergen dose used in allergy vaccines) and is able to capture the allergens before binding to their specific receptors on mast cells and basophils [47]. SIT induces IgA2 antibodies in parallel to IgG4 production that is mediated by IL-10 [48]. Moreover, the number of basophils and eosinophils is decreased in sites of allergen exposure such as skin and mucosa. Subsequently, the allergic manifestations mediated by their released cytokines (e.g., histamine, platelet activating factor, serotonin, serine proteases) subside. All of these alterations in the immune system suppress the immediate hypersensitivity symptoms and possible remodeling process in asthmatics [33,47]. SLIT and SCIT as two common routes of immunotherapy have a few differences in the way of

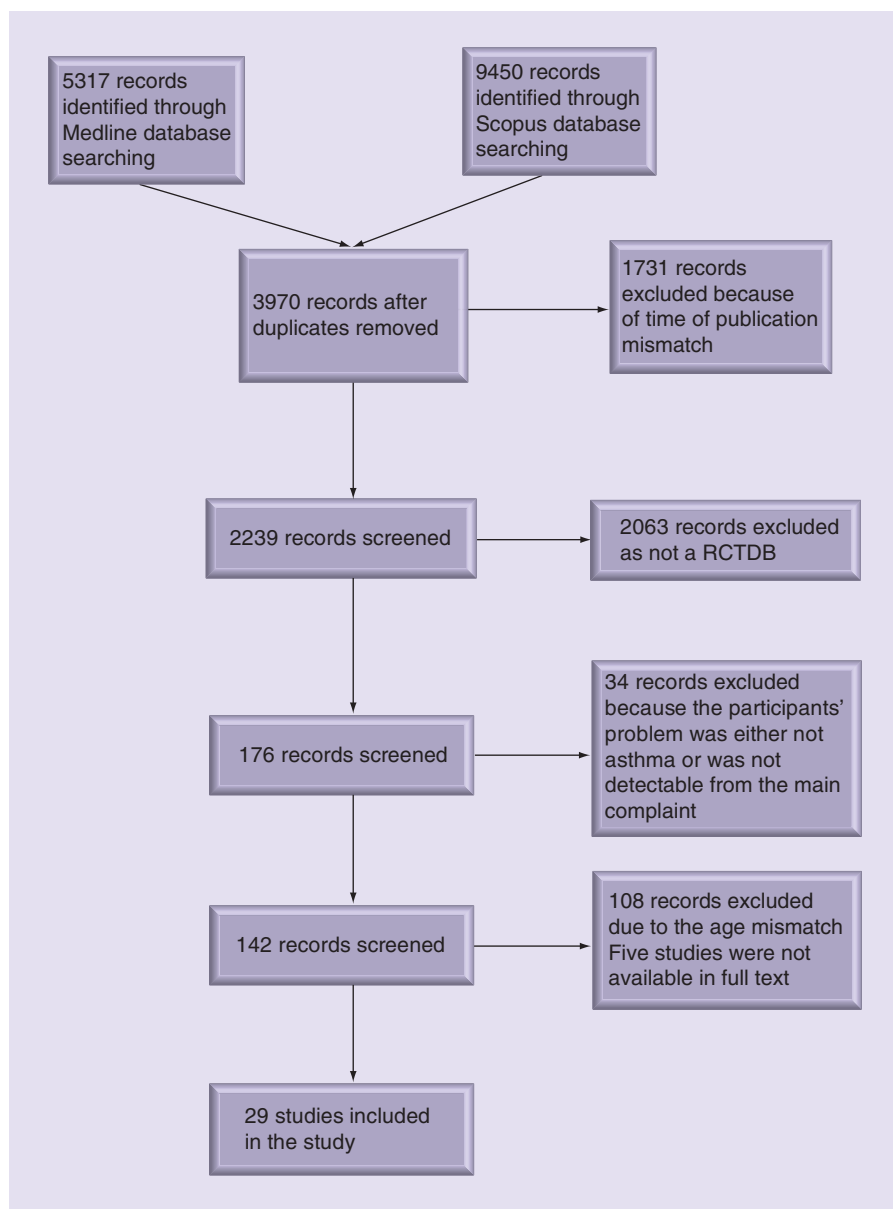


Figure 1. Included studies via primary search methodology.

RCTDB: Randomized double-blind clinical trial.

Table 1. Jadad scoring system.

Question	Answer	Score
Is the trial randomized?	Yes	+1
	No	0
Is the trial double blinded?	Yes	+1
	No	0
Has the trial reported withdrawals with appropriate description regarding cause of withdrawal?	Yes	+1
	No	0
Is the randomization method described?	Yes	+1
	No	-1
Is the blindness method described?	Yes	+1
	No	-1

tolerance induction. For successful SLIT, allergens should come into contact with oral mucosa as a critical step to be introduced to the immune system via oral Langerhans cells. Oral Langerhans cells show profound differences to their counterparts in skin in terms of higher expression of Fcε-receptor-I, MHC class I and II, and some costimulatory molecules (CD40, CD80/B7.1 and CD86/B7.2). Moreover, SLIT can elicit Th1 response via increase in IFN-γ production, but SCIT may even downregulate Th1 along with the Th2 response. On the other hand, the level of IgG4-blocking antibody is higher following SCIT than SLIT (for more details see references [49–52]).

Asthma triggers & materials of SIT: two sides of a coin

To achieve favorable results with immunotherapy, it seems mandatory to focus the procedure against the allergens that patients are sensitized to. In this regard, prior to SIT, skin prick test and/or assessment of serum specific IgE levels are usually indicated to identify target allergens [43–45,53,54]. Moreover, some patients have multiple sensitivities with different immune responses and may not benefit from single-extract SIT [55]. Polysensitized patients have significantly higher serum levels of total IgE in comparison with monosensitized subjects [56]. Bousquet *et al.* conducted a RCTDB on mono- and polysensitized subjects and showed that administration of SIT with respect to all allergens the subjects were sensitized to resulted in significantly lower clinical response in polysensitized patients [57]. Increasing the dose of extracts may improve efficacy but jeopardizes the safety of the procedure, with an increased risk of systemic reactions. To overcome this obstacle, Adkinson *et al.* used a mixture of seven allergens including HDM, ragweed grass mix, Bermuda grass, white oak, *Cladosporium herbarum*, *Aspergillus fumigatus* and *Alternaria alternata* for treatment of asthmatic children, but did not find significant benefit from immunotherapy in comparison with the control group receiving placebo [58]. On the other hand, Hedlin *et al.* found a significant decrease in bronchial hyper-responsiveness after 3 years of SIT with cat/dust mite extracts in polysensitized asthmatic children [59]. Further studies should be conducted to provide clearer evidence of the safety and efficacy of multiallergen immunotherapy [55]. A recent

review by Calderón *et al.* provides comprehensive insight to SIT in polysensitized patients [60].

Pollens

Pollens used in immunotherapeutic preparations for the treatment of asthmatic children show a wide spectrum of variability and include grass pollens such as Bermuda grass, ragweed [61,62], tree pollens [63], timothy [58,59], orchard [58,64], perennial ryegrass [58] and English plantain [58]. Fortunately, standardized extracts of many pollens including grass, tree and weed pollens that are suitable for injection or prepared in aqueous solutions (Staloral®, Stallergenes SA, Antony, France) [65] or standardized tablets (Grazax®, Abelló, Horsholm, Denmark) [66] are available in many parts of the world [67–69]. Pollen extracts are applicable using SCIT [44] or mucosal delivery via SLIT [61,62,67,68,70–72]. Children sensitized to grass pollen usually show seasonal allergic asthma and experience allergic symptoms only during this limited period of time. Airway remodeling and irreversible changes are less frequent and usually do not require long-lasting medication owing to either upper or lower respiratory tract symptoms [10]. Because of the transient nature of exposure to these allergens and the symptomatic period, the clinicians usually prescribe pollen extracts preseasonally and/or coseasonally to treat childhood pollen asthma. In the Pajno *et al.* study in 2011, symptoms, medication needs and immunological profiles of asthmatic children sensitive to pollens were compared in pre-/co-seasonal and continuous regimens via SLIT. This study showed significantly better results in the first season of treatment using continuous regimens but similar results following subsequent seasons [73]. On the other hand, in 2012 Stelmach *et al.* found no difference between pre-/co-seasonal and continuous regimens using the same materials and mode of administration in asthmatic children [74]. The classical plan of SIT includes an induction phase (build-up phase) with an updosing schedule to the maximum tolerable dose and thereafter a maintenance phase with administration of maximum dose. This procedure lasts for at least 1–3 months with longer intervals between administrations in the maintenance phase compared with the build-up phase [44]. The build-up phase usually takes 4–6 weeks and is followed by several months of the maintenance phase [75,76]. Of note, patients are more prone to show adverse events in the induction phase with increasing doses rather than maintenance phase. This warrants careful supervision, at least during the build-up period [5]. Ultra-rush immunotherapy, another challenging protocol, was examined by Stelmach *et al.* and Mösges *et al.* with maximum doses of 120 index of reaction (IR) and 300 IR of grass pollen extract achieved in only 90 min, respectively [65,71]. Increasing doses administered within a short timeframe promise maximized efficacy and better compliance; however, increased risk of systemic reactions is inevitable. Interestingly, aforementioned studies found only local adverse effects, such as sublingual itching [65,71].

Mites

HDMs, similar to other perennial allergens, elicit long-standing bronchial inflammation, airway remodeling, irreversible changes

Table 2. Double-blind randomized clinical trials comparing subcutaneous administration of different extracts in treatment of asthmatic children.

Study (year)	Jadad score	Route	Sensitive to	Protocol	Patients (n)	Associated symptoms	Age (years)	Extract/dosage	Duration (months)	Result	Side effects	Ref.
					Active	Placebo						
Pifferi <i>et al.</i> (2002)	3	SCIT	<i>Dermatophagoides pteronyssinus</i>	Classical	15	14	Rhinitis	6–14	Standardized/ up dosing to 800 U	36	Favorable	[84]
Maestrelli <i>et al.</i> (2004)	3	SCIT	<i>D. pteronyssinus</i> and <i>Dermatophagoides farinae</i>	Classical	15	8		8–16	Standardized/0.1 ml of a 0.1 BU/ml up dosing to 6 BU	36	Equivocal	[81]
Roberts <i>et al.</i> (2006)	5	SCIT	Grass pollen (<i>Phleum pratense</i>)	Classical	18	17	Rhinitis, conjunctivitis and atopic dermatitis	3–16	Standardized/ Alutard® SQ <i>P. pratense</i> up dosing to 100,000 SQ-U	24	Favorable	[187]
Ibero & Castillo (2006)	3	SCIT	<i>D. pteronyssinus</i>	Classical	15	13	Rhinitis, conjunctivitis	8–16	Standardized/ up dosing to 0.5 ml containing 42.5 µg extract	4	Favorable	[79]
Tabar <i>et al.</i> (2008)	5	SCIT	<i>Alternaria alternata</i>	Classical	13	10	Rhinitis	Mean: 14	Standardized/ up dosing to 1670 UBE with 0.1 µg extract	12	Favorable	[98]
Tsai <i>et al.</i> (2010)	3	SCIT	<i>D. pteronyssinus</i> and <i>D. farinae</i>	Classical	20	20		5–14	Standardized/0.5 AU/ml once a week (up dosing)	3	Equivocal	[83]
Zielen <i>et al.</i> (2010)	5	SCIT	<i>D. pteronyssinus</i>	Classical	33	32		6–17	Standardized/ up dosing to 0.6 ml with 1.3 µg <i>D. pteronyssinus</i>	24	Favorable	[45]
Kuna <i>et al.</i> (2011)	5	SCIT	<i>A. alternata</i>	Classical	30	20	Rhinitis, conjunctivitis	5–18	Standardized/ up dosing to 5000 TU/ml extract	36	Favorable	[43]

AU: Allergenic unit; BU: Biological unit; SCIT: Subcutaneous immunotherapy; SQ-U: Alutard SQ-unit; TU: Therapeutic unit; UBE: Equivalent biological unit.

Table 3. Double-blind randomized clinical trials comparing different allergens with placebo in treatment of asthmatic children using sublingual immunotherapy.

Study (year)	Jadad score	Route	Sensitive to	Protocol	Patients (n)		Associated symptoms	Age (years)	Extract/dosage	Duration (months)	Result	Adverse effects†	Ref.
					Active	Placebo							
Caffarelli <i>et al.</i> (2000)	5	SLIT	Grass pollen	Preseasonal	12	12	Rhinitis, conjunctivitis	5–18	Allergoid grass pollen allergens	3	Favorable	Minor local effects	[72]
Pajno <i>et al.</i> (2000)	3	SLIT	<i>Dermatophagoides pteronyssinus</i>	Classical	12	9		8–15	Standardized/ up dosing with cumulative dose of 375 µg BU	24	Favorable		[82]
Bahçeciler <i>et al.</i> (2001)	3	SLIT	<i>D. pteronyssinus</i> and <i>Dermatophagoides farinae</i>	Classical	8	7	Rhinitis	11.7 ± 3.3	Standardized/ up dosing to 100 IR/day and two-times a week in maintenance period	6	Equivocal		[102]
Marcucci <i>et al.</i> (2003; 2005)	3	SLIT	<i>D. pteronyssinus</i>	Classical	13	11	Rhinitis	4–16	Standardized/ up dosing to 0.8 and 0.4 µg main allergen	36	Favorable		[103,188]
Ippoliti <i>et al.</i> (2003)	3	SLIT	<i>D. pteronyssinus</i>	Classical	18	15	Rhinitis, conjunctivitis	5–12	Standardized/ up dosing with cumulative dose of 12 mg BU	6	Favorable		[39]
Bufe <i>et al.</i> (2004)	3	SLIT	Grass pollen	Classical	63	63	Rhinitis	9 ± 2.8	Standardized/ daily administration of drops with cumulative dose of 9.6 mg AU	36	Equivocal		[61]
Rolinck-Werninghaus <i>et al.</i> (2004)	5	SLIT	Grass pollen	Classical	39	38	Rhinitis	3–14	Standardized/ up dosing with overall three times per week and cumulative dose of 188 µg STU	32	Favorable		[62]

Favorable results indicate that statistical significant improvements in clinical measurements such as symptom scores or medication scores were observed. No systematic reaction was reported in trials depicted in the table. †Higher side effects were observed in active treatment versus placebo groups. Of note, in many studies, there is a trend to administer the conventional medications as indicated. Therefore, studies were able to report medication rescue and mean difference in medication scores after allergen-specific immunotherapy.

AU: Allergenic unit; BU: Biological unit; Ig: Immunoglobulin; IR: Index of reaction; SLIT: Sublingual immunotherapy, SQ-U: Alturad SQ-unit; STU: Specific treatment unit.

Table 3. Double-blind randomized clinical trials comparing different allergens with placebo in treatment of asthmatic children using sublingual immunotherapy (cont.).

Study (year)	Jadad score	Route	Sensitive to	Protocol	Patients (n)	Associated symptoms	Age (years)	Extract/dosage	Duration (months)	Result	Adverse effects ^a	Ref.
Niu <i>et al.</i> (2006)	5	SLIT	<i>D. pteronyssinus</i> and <i>D. farinae</i>	Classical	49 Active	48 Placebo	Rhinitis	6–12	Standardized/daily administration with cumulative dose of 2 mg IR	6	Favorable	Few minor local adverse effects [85]
Bernardini <i>et al.</i> (2006)	5	SLIT	Natural rubber latex	Classical	12	8	Rhinitis, urticaria, angioedema	4–15	Standardized/daily administration with cumulative dose of 15.3 mg	12	Favorable	[189]
Valovirta <i>et al.</i> (2006)	3	SLIT	Tree pollens	Classical	27	29	Rhinitis, conjunctivitis	5–15	Glycerinated mixture of <i>Betula verrucosa</i> , <i>Corylus avellana</i> and <i>Alnus glutinosa</i> 100,000 SQ-U/ml	19	Favorable	Minor local adverse effects [63]
Lue <i>et al.</i> (2006)	4	SLIT	<i>D. pteronyssinus</i> and <i>D. farinae</i>	Classical	10	10		6–12	Standardized/daily administration with cumulative dose of 2 mg	6	Favorable	Local adverse effects [80]
Cao <i>et al.</i> (2007)	3	SLIT	<i>D. farinae</i>	Classical	122	129	Rhinitis	4–18	Standardized/daily administration	6	Favorable	Local adverse effects [101]
Ibañez <i>et al.</i> (2007)	3	SLIT	Grass pollen	Postseasonal	45	15	Rhinitis, conjunctivitis	5–12	Standardized/grass pollen tablets (Grazax®) daily	1	Safety assessment	Pruritus and mouth edema [68]
Pham-Thi <i>et al.</i> (2007)	5	SLIT	<i>D. pteronyssinus</i> and <i>D. farinae</i>	Classical	55	56		5–15	Standardized/(Stallergenes) 100 IR tablets with 28 Ig antigen	18	Equivocal	[86]

Favorable results indicate that statistical significant improvements in clinical measurements such as symptom scores or medication scores were observed. No systematic reaction was reported in trials depicted in the table.

^aHigher side effects were observed in active treatment versus placebo groups. Of note, in many studies, there is a trend to administer the conventional medications as indicated. Therefore, studies were able to report medication rescue and mean difference in medication scores after SLIT.

AU: Allergenic unit; BU: Biological unit; Ig: Immunoglobulin; IR: Index of reaction; SLIT: Sublingual immunotherapy; SQ-U: Alturad SQ-unit; STU: Specific treatment unit.

Table 3. Double-blind randomized clinical trials comparing different allergens with placebo in treatment of asthmatic children using sublingual immunotherapy (cont.).

Study (year)	Jadad score	Route	Sensitive to	Protocol	Patients (n)	Associated symptoms	Age (years)	Extract/dose	Duration (months)	Result	Adverse effects ^a	Ref.
Bufe <i>et al.</i> (2009)	5	SLIT	Grass pollen	Pre- and co-seasonal	Active 125 Placebo 150	Rhinitis, conjunctivitis	5–16	Standardized/ grass tablets (Grazax) daily (15 mg Phl p 5, <i>Phleum pratense</i> major allergen)	9	Favorable	Local pruritus in 40 (32%) vs 3 (2%) in placebo	[67]
Stelmach <i>et al.</i> (2009)	4	SLIT	Grass pollen	High-dose ultra-shot	20	Rhinitis, conjunctivitis	6–17	Standardized/ Staloral 300 IR (<i>Dactylis glomerata</i> , <i>Anthoxanthum odoratum</i> , <i>Lolium perenne</i> , <i>Poa pratensis</i> , <i>P. pratense</i> , mean: 25 mg/ml; 10–30 to 60–120 IR) within 90 min	24	Favorable	Local, first year: 13/22 vs 5/21, second year: 7/20 vs 5/15	[65]
Mösges <i>et al.</i> (2010)	4	SLIT	Grass pollen	Ultra-rash	27		6–14	Standardized/ up dosing only within 90 min (30–90 to 150–300 IR)	2	Safety assessment	No severe reaction	[71]
Blaiss <i>et al.</i> (2011)	4	SLIT	Grass pollen	Pre- and co-seasonal	142	Rhinitis, conjunctivitis	5–17	Standardized/ once a day (<i>P. pratense</i> , 75,000 standardized quality tablet)	6	Favorable	Oral pruritus and throat irritation (70 vs 25%)	[70]

Favorable results indicate that statistical significant improvements in clinical measurements such as symptom scores or medication scores were observed. No systematic reaction was reported in trials depicted in the table. ^aHigher side effects were observed in active treatment versus placebo groups. Of note, in many studies, there is a trend to administer the conventional medications as indicated. Therefore, studies were able to report medication rescue and mean difference in medication scores after SLIT.

AU: Allergenic unit; BU: Biological unit; Ig: Immunoglobulin; IR: Index of reaction; SLIT: Sublingual immunotherapy; SQ-U: Alturad SQ-unit; STU: Specific treatment unit.

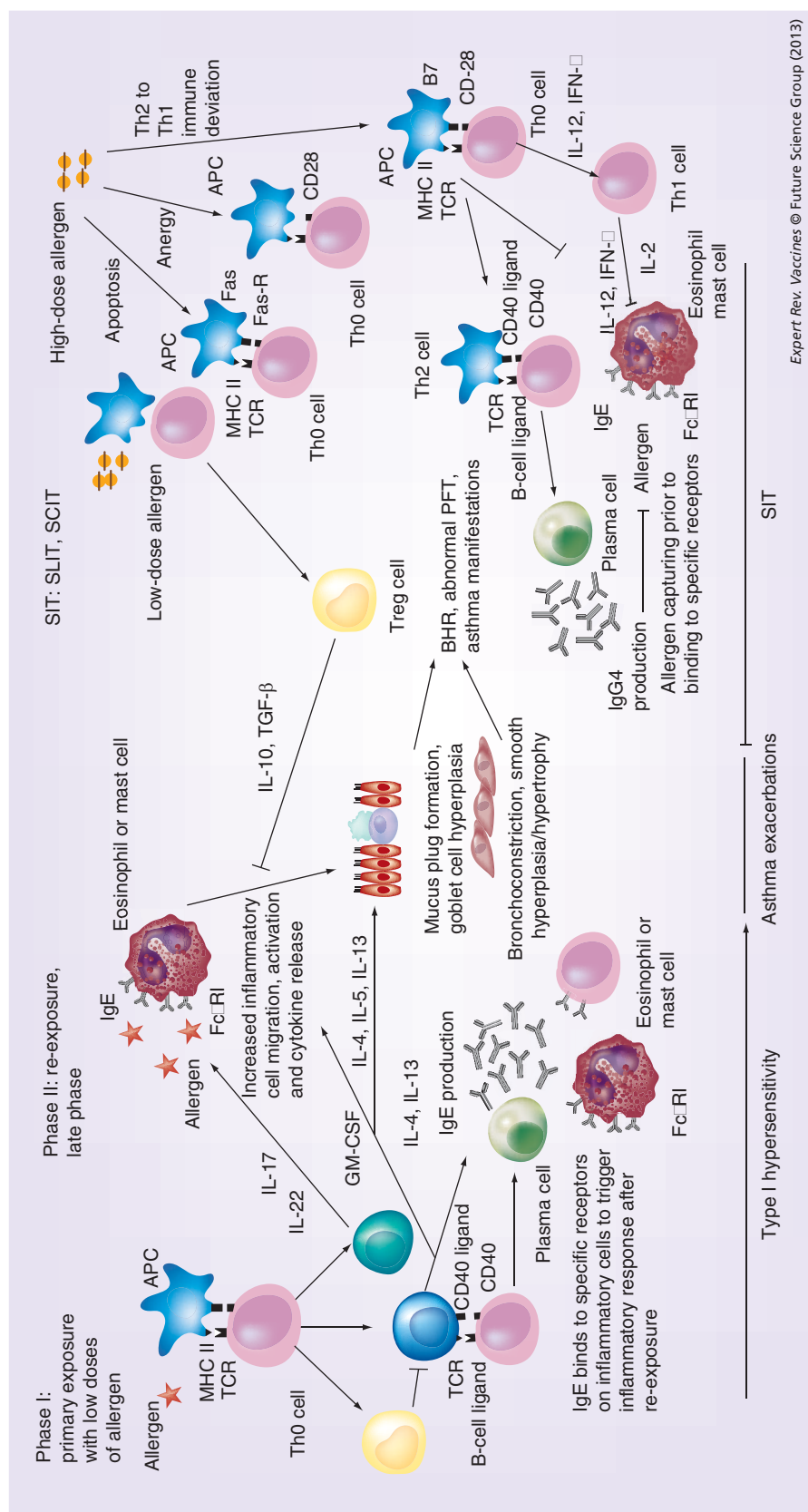


Figure 2. Hypersensitivity reactions emerge in early life after primary exposure (Phase 1) to aeroallergens. In this phase, APCs express costimulatory molecules (e.g., B7) in parallel to antigen presentation through MHC class II. T-helper differentiation is the result of this interaction, and subsequent direction is dependent on the balance between peripheral regulatory mechanisms and inflammation caused by Th2 and Th17 cells. Th2 cells stimulate B-cell maturation to plasma cells that produce IgE, and some memory B cells augment this procedure in later exposures. IgE binds to Fc ϵ RI on the surface of mast cells, eosinophils and basophils and enables them to recognize the allergen after re-exposure and cytokine production. IL-4 and IL-13 induce IgE production, mucus production in airways, goblet cell hyperplasia and smooth muscle hypertrophy and hyperplasia. IL-3, IL-5 and GM-CSF induce infiltration of inflammatory cells in the airways and IL-5 shows some direct effects on epithelial cells, goblet cells and smooth muscle cells of the respiratory tract. On the other hand, low-dose exposure to the allergens during the first doses of immunotherapy can induce tolerance by Treg activation and IL-10 and TGF- β production, which inhibits allergic reactions to the allergen. Furthermore, with high-dose immunotherapy as seen in maintenance phase, apoptosis and anergy of Th0 cells and a shift to Th1 cells can be achieved. Interestingly, after effective immunotherapy, IgEs are replaced by blocking IgG4 antibodies that capture allergens before eliciting the allergic cascade in the host.

BHR: Bronchial hyper-responsiveness; PFT: Pulmonary function test; SCIT: Subcutaneous immunotherapy; SIT: Allergen-specific immunotherapy; SLIT: Sublingual immunotherapy; TCR: T-cell receptor.

and subsequent deterioration in pulmonary function tests [10]. This evidence raises doubts about the efficacy of SIT in asthma of perennial allergens; hence, subsequent studies have evaluated SIT in mite sensitizations and, interestingly, have found favorable results [13,77]. In order to interpret the results of studies on the efficacy of each allergen extract for asthma and the employment of them in clinical practice, regional variables should be considered. While mite exposure is very high in Europe, seasonal exposures (e.g., to pollens) are more common in the USA, and this could be the reason for unfavorable results with mite extract immunotherapy in the USA compared with Europe [78]. Using the skin prick test and other alternative modalities, most of the children showed sensitivity to *Dermatophagoides pteronyssinus*, rather than other mites [45,79–83]. However, it cannot be excluded that the sets available for skin prick testing potentially are not able to detect allergy to other HDMs [84]. To administer the mite extracts, classical protocols of immunotherapy with increasing dosage were tested with minimum tolerable side effects including pain and swelling at exposition sites [45,79–83]. Standardized extracts of *D. pteronyssinus* and *Dermatophagoides farinae* suitable for SIT are available in many countries [45,79–81,85,86]. Alutard® (ALK-Abelló, SA, Madrid, Spain) for SCIT and SLITone® (SQ, ALK-Abelló, SA) for SLIT are standardized extracts of *D. pteronyssinus* and *D. farinae* [87].

Animal proteins

Cat and dog dander form another group of allergens responsible for perennial allergies and asthma but the data of SIT directing to them are very poor [88,89]. Studies involving adult asthmatic subjects provided hope to the utility of cat dander in SIT [90], but for evidence-based medicine, future studies on children are needed. Furthermore, cockroach antigens may elicit allergic reactions and development of asthma in susceptible children. An interesting study by Lin *et al.* demonstrated a significant association between sensitization to cockroach and mites including *D. pteronyssinus* and *D. farinae* in asthmatic children. Furthermore, this study suggests that with IgE levels of higher than 100 IU/ml for *D. farinae* and 0.7 IU/ml for cockroach, lower pulmonary function is seen in asthmatic children but not in children without asthma [91]. In their experience, many Taiwanese asthmatic children were sensitized to *Dermatophagoides* and cockroaches but not cat or dog dander. On the other hand, Perzanowski *et al.* found that the most common pattern of sensitization in northwest Sweden is due to birch pollens, cat and dog dander exposure and subsequent sensitization and finally development of asthma in children. According to their study, this cascade originates from schools [92]. It seems that in humid regions, mites and cockroaches are a major part of allergens that trigger sensitization, but in dry weather and far from farming environments, the role of cat and dog dander in parallel to pollens is stronger [91–94]. In a study by Srivastava *et al.*, only three asthmatic children (aged ≤18 years) were present in the active treatment group and benefited from SIT using injection of increasing doses of standardized cockroach extracts (*Periplaneta americana*) [95]. Due to the increasing evidence for the importance of animal proteins in allergic manifestations and asthma, the

literature still is awaiting prospective randomized clinical trials involving a pediatric population.

Molds

A. alternata, *A. fumigatus* and *Cladosporium* are allergenic members of the big family of molds and previous studies have demonstrated their utility in treatment of asthma either in adults [96,97] or children [43,98]. Both classical and rush protocols of immunotherapy were tested with acceptable tolerability and efficacy [96,99]. The most common pattern seen in subjects allergic to molds is polysensitization [10]. In this way, Adkinson *et al.* (as previously mentioned) studied the utility of multiallergen immunotherapy in 120 children with perennial asthma with unfavorable results [58]. However, the results should be viewed with caution owing to the strict selection criteria and assumption of rescue medication as an independent variable not a goal. Therefore, type 2 error (inability to find a significant relationship that exists in reality) could be assumed [78].

More than 20 years ago, *Trichophyton* spp. was shown to be one of the triggers of perennial asthma in adults with variable degrees of eosinophilia and bronchial hyper-responsiveness [100], but no up-to-date study exists on its role in childhood asthma or the usefulness of SIT for sensitized patients. With respect to the growing knowledge surrounding the allergens that trigger asthma, future studies are awaited to investigate the potential allergenicity of other molds and the possible chance for SIT.

Route of administration

From the introduction of SIT, different modes of administration were tested to identify the one that carries maximum efficacy and minimum adverse effects. In this way, the clinician's perspective has largely moved from subcutaneous injection towards mucosal delivery. This paradigm shift has been reflected in the literature, as over the last 20 years the sublingual route of administration has been increasingly used in studies [14].

Subcutaneous

The first report of SCIT for asthmatic children dates back to 1957 when Johnstone published his experience with 112 (72 asthmatic) children with hay fever that received ragweed pollen extracts, resulting in reduction of symptoms. For about 40 years, most of the studies focused on SCIT in the treatment of asthma either in children or adults. Unfortunately, data of RCTDBs focusing only in SCIT in the pediatric population are very poor, as shown in TABLE 2, and meta-analyses usually involve both pediatrics and adolescents altogether. All eight studies described in TABLE 2 compared medication and symptom scores between active and placebo arms. Moreover, Ibero and Castillo [79], Tabar *et al.* [98] and Zielen *et al.* [45] showed that peak expiratory flow would increase following SCIT, while Pifferi *et al.* [84] found no significant improvement in lung function tests despite improvement of asthma symptoms and decrease in asthma exacerbations. Roberts *et al.* [44] found significant improvement in cutaneous, conjunctival and bronchial reactivity to allergens along with significant improvement in medication and symptom scores. However, they did not find any

significant difference in terms of exhaled nitric oxide (an indicator of airway inflammation) between active and placebo groups. Zielen *et al.* [45] demonstrated a decrease in IgE and Kuna *et al.* [43] found an increase in blocking antibodies (IgG4) following SCIT. Zielen *et al.* also showed that SCIT leads to a reduced need for systemic steroid administration for asthmatic children [45]. In the last update of the Cochrane Database systematic review of allergen immunotherapy for asthma, only 16 out of 88 included articles focused only on children below 18 years of age and the majority of them addressed the efficacy of immunotherapy in mite asthma (nine out of 16, 57%). Overall results of this study were favorable toward immunotherapy but with better results in pollen immunotherapy rather than mite immunotherapy in which approximately two persons sensitized to pollens should receive SIT but approximately six persons sensitive to mites should be treated to avoid one deterioration in asthma [13]. On the other hand, medication score was reduced (standardized mean difference [SMD]: -0.53; 95% CI: -0.80 to -0.27) and relative risk for systemic reactions was 2.45 (95% CI: 1.91–3.13) [13].

Sublingual

SLIT has increased in popularity over the last 20 years and since the year 2000 most of researches in the field of SIT employed this route as depicted in TABLE 3. It carries out acceptable efficacy and lower risks of side effects compared with SCIT [8].

A total of 19 studies are described in TABLE 3. All of them assessed clinical response to SLIT at least in terms of symptom score, and most of them also compared medication rescue between active and placebo arms. In almost all the studies, improvement in medication score was seen with improved symptom score, while Cao *et al.* [101] found that only symptom score was improved without significant symptom score differences and Rolinck-Werninghaus *et al.* [62] found significant medication rescue without symptom score improvement following SLIT. Ippoliti *et al.* assessed the immune response following SLIT comprehensively. They evaluated serum level of Th2 cytokines, eosinophil cationic protein (ECP), prolactin, ACTH and CD40 expression at enrollment and following immunotherapy and found significant reduction in IL-13, ECP and prolactin as immune response to SIT [39]. Regarding serum-specific IgE and IgG4, Niu *et al.*, Marcucci *et al.*, Stelmach *et al.* and Pajno *et al.* found no significant difference between active and placebo arms, while Bahçeciler *et al.* found significant decrease in IgE and Bufe *et al.*, Lue *et al.*, Cao *et al.* and Pham-Thi *et al.* found significant increases in both IgE and IgG4 following SLIT [62,65,67,80,82,85,86,101–103]. Moreover, bronchial reactivity to allergens tends to decrease following SLIT [65], and lung function improves [85].

The classical plan of SLIT is usually performed by daily swallowing of drop or tablet preparations. Interestingly, maintenance dose could be administered much earlier in SLIT in such a way that SLIT could be commenced with a maintenance dose [65,71]. The allergen doses in SLIT can be up to 375–500-times higher than in SCIT [71,104]. Furthermore, SLIT could be prescribed at home with respect to the lack of a need for injections and lower risk of side effects, while SCIT should be performed under

close supervision at healthcare facilities [8,87]. SIT benefits can be observed during the first pollen season; however, SCIT has an earlier onset of action compared to SLIT [105]. SLIT is mainly accepted as an alternative to SCIT for AR in children, but to a lesser extent for asthma [106]; therefore, more evidence is needed to draw conclusions on the safety and efficacy of SLIT in childhood asthma [107].

Olaguibel *et al.* evaluated SLIT in children with respiratory allergy with a random effect size model and found that it is efficient for asthma symptom reduction (SMD: -1.42; 95% CI: -2.51 to -0.34; $p = 0.010$) and only gastrointestinal complaints were observed as a side effect in four out of 129 (3.1%) children in the active treatment group [108]. In a meta-analysis by Penagos *et al.*, nine studies that evaluated efficacy of SLIT in asthmatic children were included and showed significant reduction of symptom scores in SIT using mites (six studies) compared with those using pollens (three studies) [77]. Overall results of this study were favorable in using SLIT in either symptom reduction (SMD: -1.14; 95% CI: -2.10 to -0.18; $p = 0.020$) or rescue medication (SMD: -1.63; 95% CI: -2.83 to -0.44; $p = 0.007$) [77].

SCIT with or versus SLIT?

Eifan *et al.* (Jadad score of 5) compared SCIT with SLIT in 41 asthmatic children aged 5–10 years. A skin prick test confirmed mono-sensitivity to *D. pteronyssinus* and *D. farinae* in all of the participants; therefore an up dosing schedule of SIT was performed using 295.5 µg of *D. pteronyssinus* and 295.5 µg of *D. farinae* for SLIT and counterpart doses of 111 and 156 µg, respectively, for SCIT. They found similar efficacy for both SLIT and SCIT to relieve the symptoms and reducing medication, in parallel to decreasing serum specific IgE levels [87]. With respect to safety, SLIT was associated with no serious adverse events, while two out of 16 children who received SCIT experienced life-threatening reactions [87].

Another interesting approach was tested by Keles *et al.* (Jadad score of 3) with the combination of SCIT and SLIT in 51 asthmatic children. In their experience, SCIT and SCIT plus SLIT led to rescue asthma attacks and medication from 4 months after prescription, while in children only administered SLIT, benefits appeared after 12 months of treatment. However Treg and Th1 cytokine profiles were not significantly different among immunotherapeutic groups; only patients receiving SCIT or SCIT plus SLIT showed an increase in levels of specific IgG4 after treatment [105]. However, studies that compared SLIT with SCIT in treatment of childhood asthma are few as described, the highest level of evidence from a meta-analysis of Di Bona *et al.*'s study [109] showed that SCIT is more effective in the treatment of AR patients in terms of medication rescue (SMD: -0.58; 95% CI: -0.86 to -0.30) and symptom relief (SMD: -0.92; 95% CI: -1.26 to -0.58). It looks interesting to note that if one route of administration such as SCIT was undesirable or was associated with side effects, another route can be tested [8].

Adjuvants & conjugated molecules

The use of low doses of allergens is ineffective and high doses may lead to anaphylaxis, especially in asthmatic children who

are more susceptible to systemic reactions [110–112]. On the other hand, allergen subunits and peptides used in vaccines are not strong stimulators of the immune system. Adjuvants can improve the efficacy and safety of allergen vaccinations via the stimulation of an immune response to the minimum amount of allergens [113]. Adsorption of antigens onto the adjuvants leads to slow but prolonged release of the antigen with recruitment of APCs to the injection site (depot effect). This is also the case in encapsulating the antigens into the adjuvants. Recruitment and stimulation of APCs by persistent release of antigen triggers the cascades and results in the activation of innate and adaptive immune responses [113,114]. In addition to the immunomodulatory and delivery role of adjuvants, they are able to affect the stability of adsorbed/encapsulated antigens [115].

Aluminum hydroxide

Aluminum salts (alum) are the most common adjuvant used in national childhood vaccination programs [116]. In the context of immunotherapy, alum is able to deviate the immune response from Th2 to Th1 [117] and stimulates the activation of APCs by NOD-like receptors and its depot effect [118]. It is a safe, low-cost and efficient adjuvant with limited drawbacks [119]. Itchy nodules with local skin alterations may be seen at the injection sites and are unfavorable for children. These symptoms may last for years after injection; however, they tend to spontaneously regress in almost all cases. The rare but concerning outcome of these nodules is the development of lymphoid malignancy, so long-term follow-up has been recommended [120,121]. Despite these uncommon side effects, alum has a wide utility in SIT, especially via SCIT, for desensitization of asthmatic children [110].

Emulsions

Emulsions are two-phase adjuvants capable of boosting immunogenicity of antigens. Allergens are usually incorporated in the water phase, then the oil phase with an emulsifying agent is added to construct the vaccine [122,123]. One of the first introduced members of this family of adjuvants was complete Freund's adjuvant (emulsion with inactivated mycobacteria), which was capable of stimulating cell-mediated immunity. Despite its usefulness in stimulating the Th1 response and deviation from Th2 to Th1, its use has been forbidden not only in children, but also in adults on account of its toxicity and painful reactions at the injection site [124,125]. Incomplete Freund's adjuvant (just the emulsion without mycobacterial component) has been used in experimental studies and clinics [123]. MF59 is another squalene-containing emulsion the utility of which has been approved in influenza vaccines but not SIT [126].

Monophosphoryl lipid A

Monophosphoryl lipid A (MPL) is a nontoxic lipopolysaccharide-based adjuvant which is derived from *Salmonella minnesota* [127]. It induces a Th1-type immune response dependent on monocyte and dendritic cells and associated with a shift in favor of Th1 cytokines and a modest effect on IL-10 production. Similar to alum and emulsions, more than Toll-like receptor 4, Nod-like

receptors, especially NLRP10, play an important role in initiating the adaptive immune response in MPL-containing vaccines [128,129]. Its utility in both subcutaneous and intranasal routes was evaluated with promising results of good efficacy and safety [130,131]. The first report of treatment of allergic children (26 grass pollen and 64 tree pollen sensitized children aged 6–17 years) with Pollinex® Quattro (Bencard Allergie, Munich, Germany; an allergy vaccine that contains pollen extracts and MPL as an adjuvant) dates back to 2003 when Drachenberg *et al.* demonstrated significant improvement of medication/symptom scores following SIT in children with AR/asthma [132]. This finding was reproduced by subsequent studies [133,134]. Adverse effects related to MPL are swelling and redness at the injection site. Its safety and efficacy in childhood vaccination with attenuated toxicity has previously been shown [133,134].

Liposomes

Liposomes are lipid-based vehicles with a negatively charged bilayer structure capable of encapsulating antigens. They are spherical carriers with the capability for immunomodulation and enhancing the response to SIT. They are able to provide controlled release of their payload antigen at the target site. Moreover, modifications in lipid layers and surface of the liposomes may improve their stability, biodistribution and half-life [135,136]. Several studies showed the safety and efficacy of liposomes encapsulating *Dermatophagoides* in inducing Th1-type and strong allergen-specific IgG responses [53,137,138]. Despite all improvements, manufacturing problems and low stability limit their application in allergen-based vaccines [135].

Immune-stimulating complexes

Immune-stimulating complexes are spherical complexes of saponin, cholesterol and phospholipids with a diameter of approximately 40 nm [139]. Interestingly, they do not show a depot effect and will be rapidly removed from the injection site [140]. Their ability to provoke cellular immunity and cytotoxic CD8 T-cell responses is greater than other traditional adjuvants such as alum, and they may cause necrosis at the injection site [141,142]. This is associated with a Th1-skewed response and generation of blocking antibodies [143,144]. Despite the lytic activity of saponin and its induced hemolysis in mice [145], it is safe for human applications and its adverse effects, like local pain, are limited to injection site [142,143]. The main problem in the development of immune-stimulating complexes is related to poor antigen incorporation, which can be improved via the addition of phosphatidylcholine as a fluid lipid [139].

Oligonucleotides

CpG oligonucleotides (CpG ODN) motifs resembling bacterial DNA can bind to intracellular Toll-like receptor 9 and trigger the cascade, leading to Th1 and Treg immune responses [146]. Previous studies have demonstrated the efficacy of CpG ODN in prevention of airway remodeling in animal models of asthma [147]. It is worth noting that it is effective in reducing manifestations of asthma via intranasal, inhalation and even oral

administration [148]. Despite promising results in animal studies and clinical trials on adult AR/asthma patients, in an interesting study by Nayak *et al.*, administration of Amb 1 CpG ODN (AIC) to children sensitized to ragweed resulted in nonsignificant improvements in symptoms and rescue medication compared with the children who received placebo. Furthermore, serious adverse events were not observed [149]. Further studies are awaited to find the possible benefits of AIC vaccines; however, existing data on the immunomodulatory effect of CpG ODNs promise hope to provide more effective treatments for asthma [147,148,150].

Polymeric nanoparticles

Nanoparticles provide a subset of adjuvants in the scale of less than 1 μm , which is within the range of antigen-presenting systems in human body [151]. Polymers concomitant with antigens, or even alone, have immunomodulatory effects [152]. In general, they boost immunogenicity of loaded antigens via the following mechanisms:

- Enhancement of antigen uptake by APCs, activation and maturation of dendritic cells [152];
- Priming of T cells and proliferation of allergen-specific memory CD4⁺ T cells [153];
- Stimulation of production of TNF- α , IL-1 and IL-8 [153];
- Amplification of innate immune response and activation of adaptive immune response in favor of Th1 and production of blocking antibodies [154].

They include biodegradable and nondegradable adjuvants capable of providing a controlled/sustained delivery system of antigens in the target. They protect the loaded antigen from degradation by enzymatic and pH conditions in the GI tract, and facilitate the uptake of antigen via the mucosal-associated lymphoid tissue. These properties make nanoparticles a good candidate for oral and intranasal administration of vaccines, which is accompanied by better patient compliance [151,155].

What is the role of mycobacteria and probiotics in allergen immunotherapy?

Mycobacteria

Immunomodulatory effects of mycobacteria *per se* might not prevent from asthma. On the other hand, BCG vaccination does not predispose children to develop asthma or atopic symptoms [156]. Furthermore, concomitant BCG administration as an adjuvant to the allergy vaccine may improve SIT efficacy. This idea was supported by studies on animal models of asthma which showed a shift toward Th1-type cytokines and enhancement of the cellular immunity [157–159]. In clinical practice, Arkan *et al.* found no difference between children who received BCG as an adjuvant and those who were treated only with *D. pteronyssinus* extract [160]. Similarly, Cohon *et al.* showed no benefit from the addition of BCG to SLIT of asthmatic children. In fact, in both stated studies, BCG only resulted in a significant increase in IL-12 and IL-10 levels, without any benefit in relief of atopic symptoms [161]. Interestingly, Ou-Yang *et al.* developed a recombinant BCG vaccine which expressed Der

p2, and demonstrated its efficacy in the modulation of the immune response in the favor of Th1 type and the reduction of inflammation in sensitized mice [162].

Probiotics

Probiotics are live microorganisms with the ability to confer a health effect on the host when consumed in adequate doses [163]. Lactic acid bacteria and *Bifidobacterium* strains are well-known probiotics and are present in high amounts in the intestinal flora of healthy children, while they are reduced in allergic children [164]. It is understood that the gut microbiota is associated with normal maturation of the immune system [165]. On the other hand, probiotic bacteria can modulate immune responses in favor of Th1 even in the presence of allergens such as HDMs and *D. pteronyssinus* [166]. These findings suggest that modulation of the immune system by probiotic administration can be helpful in atopic asthmatic children. Two studies by Giovannini *et al.* [167] and Stockert *et al.* [168] in 2007 showed no significant benefit from probiotic adjuvants of *Lactobacillus casei* (10^{10} CFU) and *Enterococcus faecalis* (18×10^7 CFU) to specific asthma immunotherapy in children younger than 12 years of age. Another study by Rose *et al.* confirmed the aforementioned results with respect to the application of *Lactobacillus rhamnosus* (10^{10} CFU) as a target probiotic strain [169]. In this regard, Van Overtvelt *et al.* examined 11 probiotic strains including *Bifidobacterium*, *Lactobacillus* and *Streptococcus* strains and introduced *Lactobacillus helveticus* as an efficient immune modulator and a factor capable of reducing the hyper-responsiveness and bronchial inflammation in a murine model of asthma [170]. Further studies should be conducted to evaluate other probiotic strains and the appropriate dosage to clarify the possible utility of probiotics in asthmatic children.

Modifications in routine SIT procedure: advantage or disadvantage?

Recombinant allergens, peptide immunotherapy & DNA vaccination

As a routine, preparations for SIT are extracted from natural allergens – either herbal/fungal or animal sources. Presence of nonrelevant materials that are useless or even have a negative impact on the results of SIT is inevitable [171,172]. To overcome these obstacles and produce pure, consistent and more potent allergens, technology of recombinant allergen production has been employed [14]. Furthermore, this enables researchers to find new and more relevant allergen derivatives and provide complete panels to detect any source of allergic reactions with a high accuracy [171,172]. In this way, individualized immunotherapeutic protocols with specific utility in polysensitized subjects can be developed [173]. There were promising results with the use of recombinant allergen immunotherapy in adults sensitized to the pollens; however, data on the safety and efficacy of this approach in the pediatric population are lacking [174].

Similar to recombinant allergens, peptide immunotherapy relies on short sequences of T-cell epitopes of corresponding intact allergen with maintained immunogenicity alongside a reduction in

allergenicity [175]. However, technology for reaching the cloned proteins produced by appropriate hosts such as *Escherichia coli* is expensive and sometimes outstanding from the practice, synthesized peptides are more easily produced and standardized [175]. With the employment of more pure peptide structures, clonal expansion of T and B cells following re-exposure to the allergen might be decreased [176]. Because these peptides lack tertiary configurations, they are unable to activate IgE-producing cells, but tolerance induction can be achieved with only the primary structure of the main part of the allergen [14,177].

DNA vaccines consist of DNA sequences encoding the target peptide that are incorporated into plasmids. APCs take up the plasmids after administration and the target peptide can be synthesized by the receiver's APCs. Subsequent cascades are triggered, similar to the administration of synthesized or recombinant peptides [175,178].

Anti-IgE antibodies & antileukotrienes

Administration of anti-IgE antibodies provides nonspecific blockage of specific IgE related to allergic reactions. When omalizumab was administered as an anti-IgE during SCIT to children sensitized to birch and grass pollens, it resulted in a significant reduction in leukotriene release during allergic seasons [179]. With respect to the clinical outcomes, pretreatment prescription of omalizumab was associated with increased rescue medication in asthmatic adults during SIT [180]. Furthermore, Kopp *et al.* demonstrated a significant decrease in symptom scores without rescue medication following cotreatment with omalizumab in SIT. In contrast, montelukast (antileukotriene) treatment during SIT was shown to cause increased asthma exacerbations in children [181]. These controversial results warrant further powerful studies to demonstrate the exact role of anti-inflammatory drugs in SIT.

Expert commentary

Utilization of allergen extracts as vaccines capable of downregulating the destructive immune response and ameliorate the related symptoms, opens insights into the treatment of allergic diseases. Asthma in childhood often has an allergic basis with a trend towards persistence into adult life that justifies a curative treatment. Modification of the natural course of disease during childhood rather than adult life is more cost effective compared with available symptomatic treatments. Moreover, SIT can prevent new sensitization and asthma development in children with AR. First evidence regarding this preventive role of SIT was raised from Johnstone and Dutton's study on allergic children and confirmed by subsequent studies such as the PAT study [182]. The PAT study revealed that after 3 years, allergic children treated with SIT were more likely to be free of asthma compared with those who received placebo, with an odds ratio (OR) of 2.52 [23,183]. Similar results were reproduced by Novembre *et al.* in such a way asthma development was 3.5-times higher (95% CI: 1.5–10.0) in children with hay fever who received standard symptomatic therapy compared with those who received SLIT [23]. Regarding new sensitization, Des Roches *et al.* found that from 22 children who received SIT, nobody developed new sensitization, while all HDM-sensitized

patients who were treated with standard symptomatic therapy had new sensitization after 3 years ($p = 0.001$) [16]. Longer duration of follow-up with more participants confirmed this protective effect of SIT. Pajno *et al.* followed 123 children monosensitized to HDM for 6 years and found that 52 out of 69 children in the SIT group compared with 18 out of 54 children in the control group were still monosensitized (conferring an OR of 6.1 for prevention from new sensitizations) [18]. Inal *et al.* followed 147 children monosensitized to HDM for 5 years and found that 64 out of 85 children who received SIT compared with 29 out of 62 children in standard symptomatic therapy did not develop new sensitization at the end of study (conferring an OR of 6.6 for prevention from new sensitizations) [16–19]. This could suggest that there is a close relationship between AR and asthma and they may be two manifestations of one disease [23,183]. It is also suggested that tolerance induction is implicated in reprogramming the immune response of an AR child and prevents not only asthma development but also new sensitizations [184]. Through the SIT procedure, inappropriate Th2 deviation can be corrected with shifting to Treg or Th1 cells and apoptosis or anergy of Th0 cells that can be seen with high-dose regimens. Subsequently, immune mediators show a significant decrease in specific IgE antibodies and increase in IgG4 antibodies that block the trigger of asthma after re-exposure. To achieve the desired results, selection of appropriate materials, route of administration and pre-/comedication is required and sufficient information regarding different aspects of SIT and its immunomodulatory effects is necessary. Initially, SIT with pollen allergens via subcutaneous injection was investigated; afterward, studies focused on the safety and efficacy of SIT with other allergens and alternative modes of administration such as the sublingual route. To administer allergy vaccines, both SLIT and SCIT are associated with favorable results; however, SLIT with no need for injection, low risk of anaphylaxis and possibility of use at home instead of healthcare facilities, seems to be a better route in pediatrics.

Generally, there is considerable heterogeneity among preparations of allergy vaccines worldwide. Most preparations in Europe are single-allergen, while in the USA, multiallergen formulation is more common [185]. Furthermore, preparations may be pure unmodified, modified with chemical materials (glutaraldehyde or formaldehyde) or adsorbed (depot extracts) or even recombinant. This variability is also noticeable in concentrations of allergen extracts and the units used to express them [8].

New generations of allergy vaccines such as MPL-containing allergy vaccines, also named Pollinex Quattro, showed safety and efficacy in asthmatic children in postmarketing evaluations [133]. However, higher levels of evidence are needed to claim usefulness of allergy vaccines with new adjuvants rather than alum in children. Unfortunately, there are few publications that assess efficacy and safety of new allergy vaccines in pediatric populations. Any change in pre-/co-medications to improve the SIT efficacy or safety could work, but should be employed with caution owing to the poor available evidence. Overall, SIT seems to be effective and safe for asthmatic children that meet its indication.

Five-year view

Future studies with a large number of participants should be conducted to find the answer of the question: who will benefit from SLIT or SCIT? The answer should be defined with respect to the age, associated disease and the type of sensitivity of the asthmatic children. Of note, the risk of side effects should be balanced with the efficacy and the onset of benefits; the treatment protocols and duration of treatment should be determined carefully to reach an optimized, cost-effective approach, and further studies with multi-allergen immunotherapy are awaited. Nevertheless, with respect to the fact that SIT is the only available curative treatment for allergic respiratory disease, it will be used more in the next 5 years. This claim is based on the growing knowledge on SIT and the increase in prevalence of allergic disease throughout the past decade. Additionally, novel allergy vaccines will be introduced with better safety and efficacy profiles. Newer routes of administration like intralymphatic immunotherapy (ILIT) and modular-antigen translocation (targeting MHC class II) as new strategies of vaccination may be investigated in SIT of asthmatic children. Recently, the first trial on ILIT targeting the MHC class II in patients allergic to cat allergens revealed that this approach was safe and

resulted in tolerance induction after ILIT was performed only three times [186]. Of note, none of the participants was younger than 18 years of age and results were not reported on asthmatics separately. The future of allergy vaccines will be based upon first vaccines with new adjuvants such as CpG ODNs, MPL and nanoparticles, and on recombinant vaccines. This change in formulation of allergy vaccines will be reflected in the schedule of SIT, like ultra-rush courses for administration of Pollinex Quattro rather than classical long periods of SIT with Grazax® (Abelló, Horsholm, Denmark). Shorter courses of immunotherapy are associated with better compliance of patients, which is of utmost importance in children. Accordingly, in the next 5 years, SIT will be one of the best options for an atopic asthmatic child worldwide.

Financial & competing interests disclosure

The authors were supported by a grant from the Tehran University of Medical Sciences (91-03-30-19248). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Asthma in children usually has an allergic basis, with a Th2-biased response and enhanced IgE.
- Allergen-specific immunotherapy (SIT) is the only available curative treatment toward the basis of the disease. The main mechanism behind a successful SIT course is the tolerance induction.
- Allergy vaccines can be administered via sublingual immunotherapy (SLIT) or subcutaneous immunotherapy. SLIT could be prescribed at home, with no need for injection and a lower risk of side effects compared with subcutaneous immunotherapy. Hence, SLIT seems a better route of administration in children.
- The efficacy of SIT in monosensitized patients is established, while in polysensitized patients, the best schedule of SIT still needs to be established.
- Alum is widely used as an adjuvant of allergy vaccines but the future of adjuvants lies in monophosphoryl lipid A, CpG ODNs and nanoparticles.
- Addition of other products such as probiotics, anti-IgE antibodies and antileukotrienes was not shown to improve the efficacy of SIT.
- SIT with recombinant allergy vaccines omits any nonrelevant materials and provides a more specific treatment as compared with other popular vaccines.
- Despite all the improvements in SIT, more studies in asthmatic children regarding the best vaccine formula and schedule of administration are needed. Long-term follow-up of treated children is also awaited.

References

Papers of special note have been highlighted as:

• of interest

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