

# Allergen immunotherapy for allergic rhinitis \*

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## Summary

Allergic rhinitis, a risk factor for bronchial asthma, is a global health problem that impairs patients' physical and social activity and consequently their quality of life. Specific Immunotherapy (SIT) involves the administration, subcutaneously or sublingually, of increasing doses of the causative allergen, in order to induce clinical and immunologic tolerance. SIT has been shown to be effective in those with a poor response to conventional drug therapy. Immunotherapy has been shown to have disease-modifying effects and result in long term remission of allergic symptoms and reduces the risk of progression from rhinitis to asthma, as well as the chances of developing new sensitizations to allergens. Injection immunotherapy is a safe treatment for allergic rhinitis with/without mild controlled asthma, provided that it is performed in the context of a harmonious interaction between trained medical personnel and appropriately selected patients. Immunotherapy suppresses early and late responses to allergen exposure by modifying both T-cell and B-cell responses to inhaled allergens. Immune deviation of allergen-specific T cell responses in favour of Th1 and/or the induction of regulatory T cells is crucial in achieving immune tolerance. Increased understanding of the mechanisms of immunotherapy has identified potential biomarkers of the response to treatment and highlighted new therapeutic pathways with potential for even more effective future standardized vaccines.

**Key words:** allergic rhinitis, IgE mediated allergy, allergen immunotherapy, immune tolerance, T regulatory cells

## Introduction

Allergic rhinitis represents a major consequence of IgE sensitization against common inhaled aeroallergens. Depending on the type of sensitization and geographic considerations, symptoms may be seasonal or perennial. According to the ARIA classification, symptoms are classified according to duration (intermittent or persistent) and severity as mild or moderate-severe, the latter implying a significant impact on life quality of the sufferer. The prevalence of atopic sensitization in westernized countries is approaching 40 – 50% of the general population, the majority of whom will express allergic symptoms, including those of allergic rhinitis<sup>(1-4)</sup>. Consequently, allergic rhinitis is a global health problem that impairs patients' physical and social activity. Typical symptoms include intense nasal itching, sneezing, anterior watery discharge and nasal obstruction, anterior watery nasal discharge and sneezing. Allergic rhinitis is very commonly accompanied by conjunctivitis and is a risk factor for co-morbid

bronchial asthma. Treatment strategies include allergen avoidance where feasible, appropriate pharmacotherapy and, in carefully selected patients who fail to respond to these measures, allergen specific immunotherapy (SIT).

SIT was first performed a century ago<sup>(5)</sup> by Noon and Freeman at St Mary's Paddington in London, UK. The first randomized controlled trial was completed by Frankland and Augustus in 1954 in the same hospital<sup>(6)</sup>. Since then, considerable research has contributed to elucidating the underlying mechanisms and establishing the place of immunotherapy in treatment, novel treatment routes and immunotherapy strategies. SIT is the only known treatment to alter the natural course of allergic disease<sup>(7-9)</sup>.

SIT involves the repeated administration of gradually increasing quantities of specific allergen extracts-vaccines in order to raise the patient's tolerance to the offending allergen. SIT, as an option, depends on careful evaluation of the patient's profile

according to disease severity, type, efficacy of usual anti-allergic drugs, consideration of potential adverse events and effectiveness or otherwise of allergen avoidance measures<sup>(10)</sup>.

## PRACTICAL ASPECTS

The classical route of immunotherapy administration is subcutaneous injection in the upper outer arm (SCIT). The allergen extract is a biological product, which usually contains physically modified allergen extract. The most commonly used carrier in Europe is aluminum hydroxide, which provides sustained release of the allergen from the injection site, a depot effect. There are two phases of SCIT administration, the initial, build-up phase when the dose and concentration of the vaccine is slowly increasing generally at weekly intervals for three to four months. There follows a maintenance phase, lasting 3-4 years, when the optimal therapeutic dose has been achieved and thereafter repeated at monthly intervals for the rest of the therapy. Of course there are variants of SCIT schedules regarding duration of the phases, doses and concentrations depending on the manufacturer. The patient has to remain in physician's office for at least 30 minutes after injections (60 min in UK) in view of the small risk of systemic allergic reactions to the vaccine.

Recently, at least in Europe, the sublingual route has emerged as a popular alternative to subcutaneous immunotherapy (SLIT). The patient retains the fluid or a tablet under the tongue for two to three minutes and then swallows. SLIT is associated with either a short or no initial up dosing phase and the maintenance dose is self-administered at home, generally on a daily basis. The recommended duration of either SCIT or SLIT is 3 to 5 years.

## PREREQUISITES

### Patient selection

Patients with allergic rhinitis require a detailed history, physical examination and selected tests to define IgE sensitization to aeroallergens. Skin prick testing (SPT) is the preferred method while in vitro testing for allergen-specific IgE antibodies is also an useful effective alternative. Prior to SIT the following prerequisites need to be addressed:

- Clinical relevance: It is essential to relate the expression of symptoms following the relevant allergen exposure. In this clinical context the SPT/ or RAST test provides an objective confirmation measurement of IgE sensitivity to the offending allergen correlating with symptoms onset and the timing of the relevant allergen exposure<sup>(11,12)</sup>. Importantly, a positive SPT alone confirms only IgE sensitization and in the absence of symptoms on relevant exposure does not necessarily mean allergic disease.
- SIT is a particularly viable option in allergic rhinitis alone or when it is accompanied by mild asthma on relevant allergen exposure that is stable and associated with normal or near normal lung function (FEV1  $\geq$  80% predicted). The

prevalence of asthma in patients with rhinitis varies from 10% to 40%<sup>(13-16)</sup>. In contrast, the large majority of asthmatics suffer from co-morbid allergic rhinitis confirming the well-known concept of 'one airway one disease' (ARIA)<sup>(17)</sup>.

- Monosensitized individuals are strong candidates for SIT. On the other hand, recent studies have shown that treatment with a single allergen, although specific for that allergen, may be equally effective in polysensitized patients – the important determinant being that the dominant association of symptoms is with the allergen used for treatment with little/no symptoms or requirement for rescue medication in relation to other allergens giving rise to IgE sensitization<sup>(18-20)</sup>. Therefore it is crucial to identify those clinically relevant allergens in polysensitized patients. SIT administered as two or three unrelated allergen extracts given separately is common practice but requires formal testing in clinical trials<sup>(21)</sup>.
- Immunotherapy is contra-indicated in patients with moderate-severe, uncontrolled asthma, autoimmune, malignant and cardiovascular diseases.
- Pregnancy: Immunotherapy should not be initiated in pregnancy whereas if well-tolerated, maintenance immunotherapy may be continued during pregnancy.
- Age of onset: Current guidelines recommend immunotherapy in children above the age of five years. This recommendation is empirical and in part based on the difficulty of recognizing early symptoms and signs of anaphylaxis below this age. There is no upper age limit as long as other chronic diseases do not coexist.

Measures of efficacy are mainly clinical parameters that include symptom, medication score<sup>(22)</sup> and measurements of quality of life. Life quality evaluation may be disease specific<sup>(23)</sup> or a general assessment<sup>(24)</sup>.

### Allergen selection

Efficacy has been identified for the following allergens<sup>(17)</sup>:

- Pollens<sup>(25-30)</sup>: Grasses, Parietaria, Olive, Cypress, Birch (and highly cross reactive Alder, Hazel), Ragweed,
- House dust mites<sup>(31,32)</sup>: *Dermatophagoides pteronyssinus*, *Farinae*,
- Animal epithelia<sup>(33,34)</sup>: Cat.

Preliminary data is available for immunotherapy for dog<sup>(34)</sup>, cockroach<sup>(35)</sup> and mould allergy<sup>(36,37)</sup> whereas at present there is insufficient information to recommend their routine use outside clinical trials.

### Adherence to immunotherapy

A major issue is adherence to complex and prolonged treatment regimens - the patient has to be well-informed about the duration and the frequency of allergen administration prior to initiation of immunotherapy. Studies regarding SIT adherence

in the literature are lacking but the rate seems to be 70-75%<sup>(38)</sup> because of inconvenience, not immediate symptom relief, costs and loss of working hours. Patient characteristics associated with SIT adherence have been reported and include age, gender, race, presence of comorbid asthma, severity of disease, education level and social status<sup>(39)</sup>. Clinical experience has shown that a detailed and thorough update of the patient increases the likelihood of adherence to SIT protocols.

### EFFICACY

Measures of efficacy of SIT are mainly clinical parameters that include symptoms, medication scores<sup>(22)</sup> and measurements of quality of life. Life quality evaluation may be disease-specific<sup>(23)</sup> or a general assessment questionnaire<sup>(24)</sup>.

### SCIT

Many randomized double-blind placebo controlled clinical trials (R DB PC) demonstrate a beneficial effect of SCIT in children and adults suffering from allergic rhinitis and asthma caused by different allergens<sup>(40-48)</sup>. Meta analyses of SCIT efficacy have strongly confirmed these results<sup>(25,49-51)</sup>. SCIT is effective in moderately severe seasonal allergic rhinitis, including patients who give a history of failure to respond to antihistamines and nasal steroids. Across the whole season, mean symptom and medication scores were 29% and 32% lower compared with the placebo group<sup>(18)</sup>.

When SCIT for grass allergy was given for 3 to 4 years, symptom medication scores remained low for at least 3 years after the randomized controlled withdrawal of treatment<sup>(9)</sup>. This longterm benefit was not observed when duration of immunotherapy was limited to only one year<sup>(52)</sup>. Other studies suggest that persistent clinical benefits may persist even ten years after discontinuation and these results contrast sharply with conventional medication<sup>(53,54)</sup> which provides no benefit after discontinuation. Generally, clinical symptoms need to be re-evaluated annually and, in case of insufficient clinical response, the patient should be re-assessed regarding their profile of symptoms on allergen exposure in relation to their IgE-sensitizations.

SCIT might prevent the evolution of rhinitis to asthma<sup>(7,55-58)</sup>. The preventive allergy treatment (PAT) study showed that SCIT can reduce the development of asthma 2-3 fold in children 6-14 years old with allergic rhinoconjunctivitis. Three years after discontinuing therapy only 26% of the SCIT treated patients demonstrated asthma in comparison to 45% of the control group<sup>(7,55)</sup>. This level of protection has now been confirmed to last up to 10 years after initiating a 3 year course of therapy<sup>(59)</sup>. Though the evidence is less robust, SIT might also prevent the development of new allergen sensitivities in monosensitized patients<sup>(43,60,61)</sup>.

### SLIT

There are many randomised placebo-controlled trials, many

Table 1. Separate Cochrane meta-analyses of symptom and medication scores for SLIT and SCIT<sup>(93,94)</sup>.

	SCIT		SLIT	
	SMD	95% CI	SMD	95% CI
Symptom score (cf placebo)	-0,73	-0.97 to -0.50 (P < 0.00001)	- 0,49	-0,64 to - 0,34 (P < 0,00001)
Medication score (compared to placebo)	-0,57	-0.82 to -0.33 (P<0.00001)	- 0,32	-0,43 to - 0,21 (P<0,00001)

with extracts of grass pollen, that show that SLIT is effective for allergic rhinitis<sup>(18,20,49,62,63)</sup>. Meta-analyses of efficacy have strongly confirmed these results for SLIT<sup>(70-73)</sup>. A meta-analysis of SLIT in children was equivocal with considerable heterogeneity, whereas more recent large clinical trials with grass allergy tablets for sublingual use demonstrated that SLIT in the pediatric allergic rhinitis population showed comparable levels of efficacy as observed in adult populations<sup>(64-66)</sup>. Data for SLIT efficacy against house dust mites are not sufficient<sup>(67)</sup>. Large SLIT clinical trials need to be run regarding allergens other than for grass pollen<sup>(68)</sup>. Long-term clinical improvement indicating disease modification has also been shown for at least 2 years following 3 years of grass pollen sublingual immunotherapy<sup>(69)</sup>. Evidence for SLIT preventive effects regarding the potential appearance of asthma is also available although currently less convincing<sup>(8,70,71)</sup> and further studies are in progress.

### SAFETY

#### SCIT

SCIT involves the injection of allergens in an IgE-sensitized patients, such that inevitably SCIT carries a low but definite risk of inducing systemic allergic reactions<sup>(72,73)</sup>. Frequency and severity of adverse effects vary in different studies depending on the allergen product, initial phase protocol, allergy severity especially in asthma patients, inclusion and exclusion criteria. Very rarely anaphylaxis may occur, which highlights the importance of performance of immunotherapy only by those trained and experienced in the early recognition of systemic allergic symptoms and in the performance of immunotherapy according to published guidelines. Two people, including one physician and facilities for resuscitation should be immediately present at all times and patients kept for observation for at least 30 min after injections (60 min in UK).

Systemic reaction prevalence to SCIT ranges from 1% to as high as 35% of patients receiving rush protocols of immunotherapy<sup>(74,75)</sup>. Generally, rush protocols are not used in patients with allergic rhinitis and confined to patients with insect venom allergy. In a retrospective study in Italy over 20 years, there were 115 systemic reactions in 435,854 injections to 4,000 patients. The

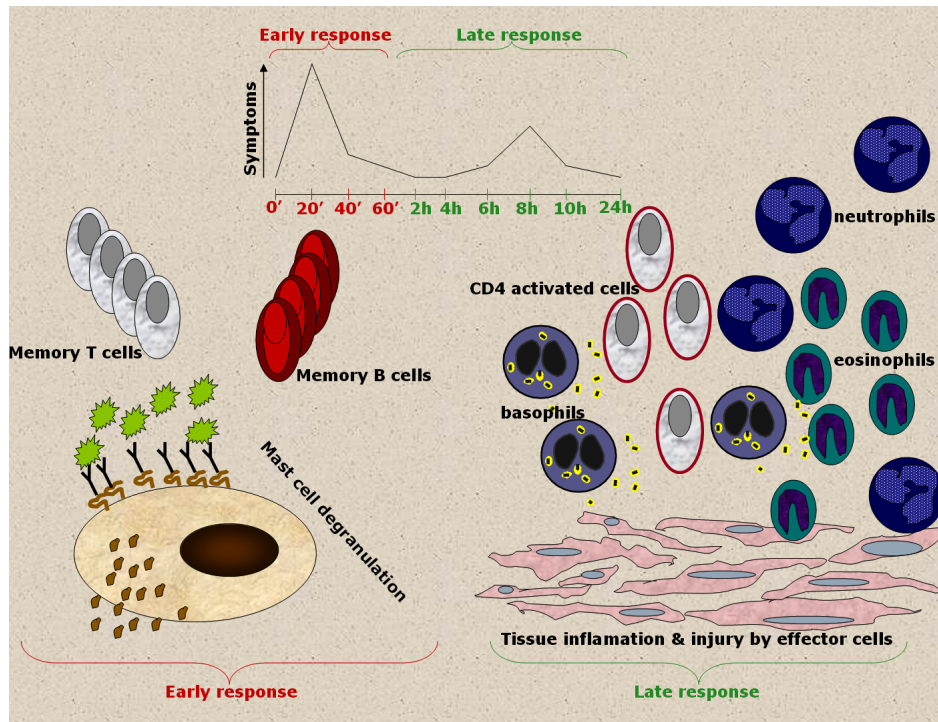


Figure 1. Phases of the allergic reaction.

results show improvement in terms of SCIT safety between two decades<sup>(76,77)</sup> (Table 2). In a multicenter prospective study there were 53 reactions out of 17526 injections (0,3%), in 18 patients out of 423 patients (3,7%)<sup>(78)</sup>.

In an American Academy of Allergy asthma and Immunology survey, the incidence of near fatal reactions was 5.4 per million injections<sup>(79)</sup>. Half of the respondents highlighted that anaphylactic reactions occurred more commonly during the pollen season and one fourth as a consequence of immunotherapy dosing errors. There were 41 fatalities, the estimated rate being 1 per 2,5 million injections. Presence of asthma was the major associated risk factor in most of the cases. The most common near fatal reaction was respiratory failure and 57% of these patients had a baseline FEV1 of less than 70% of the predicted value. Administration during the peak pollen season and the occurrence of previous systemic reactions were cited also as major contributing risk factors. Thus an assessment of patient's current health status is mandatory before the administration of injections, especially asthma status, including an obligatory measurement of airflow obstruction by peak flow meter use. Identification of risk factors will reduce the possibility of a systemic reaction and the immediate availability of appropriate resuscitation equipment is mandatory<sup>(26,80,81)</sup> (Tables 3, 4).

The vast majority of systemic reactions to SCIT occur within 30 minutes after injection<sup>(82)</sup> and this is particularly so for more severe reactions. Literature review showed that in 70% of reactions the onset after the injection was less than 30 minutes<sup>(75)</sup>. The European Academy of Allergy and Clinical Immunology strongly recommends a wait period of 30 minutes in the physi-

cian's office<sup>(83)</sup>. In the UK this recommended period is one hour<sup>(80)</sup>. Epinephrine intramuscularly is the treatment of choice and antihistamines, corticosteroids are secondary medications that might help to modify a systemic reaction. Consequently, use of beta-adrenergic blocking agents are a contraindication SCIT, since they prevent adrenaline therapeutic actions. Absolute and relative contraindications are given in Table 5<sup>(84)</sup>.

Local side effects are very common with a frequency ranging from 26% to 86% of injections although in general well-tolerated and not requiring treatment<sup>(85)</sup>. A major issue is whether a local reaction predicts a systemic one. Two retrospective studies approached this question comparing the effect of not modifying immunotherapy dose based on local (early and/or late phase) reactions (12,464 injections) with dose adjustment schedules (9,542 injections)<sup>(85,86)</sup>. There was no statistical difference between these two protocols regarding systemic reactions. On the other hand, increased frequency of large local early phase reactions (>25 mm) in a patient might predict a greater risk of a systemic reaction<sup>(87)</sup>.

### SLIT

SLIT is better tolerated than SCIT, providing greater safety and this seems to be its major advantage since it permits self-administration without medical monitoring. A review of 66 studies representing 4,378 patients, who received 1,181,000 doses<sup>(88)</sup>, showed local oral mucosal reactions to affect up to 75% of patients usually in the beginning of therapy and thereafter being well-tolerated. Systemic reactions occurred in 169 out of 314,959 doses (0,056%). The majority of these reactions referred

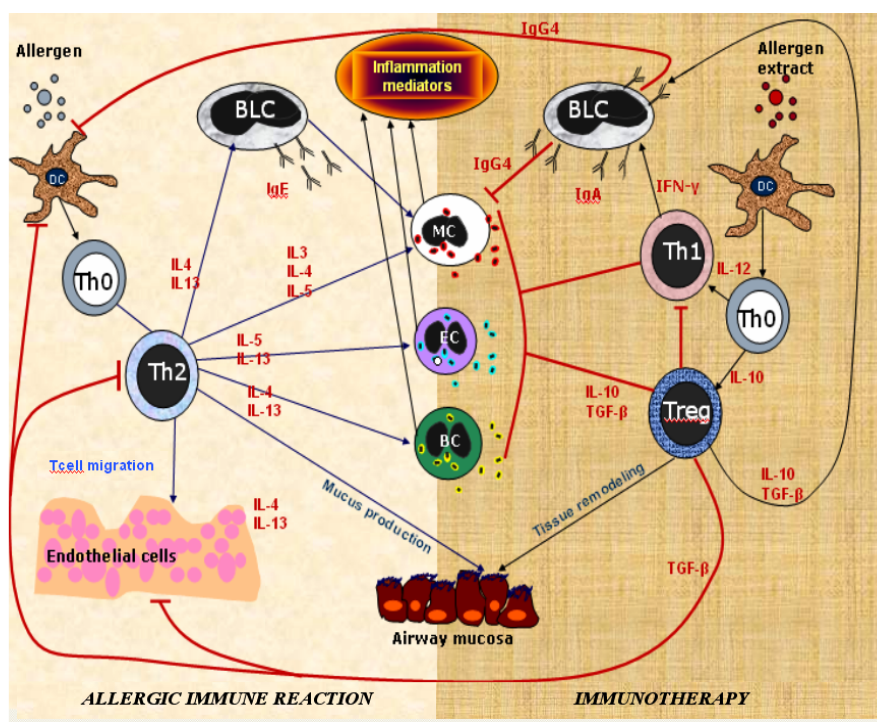


Figure 2. Contrast image of allergic immune reaction and immunotherapy. Contrast of allergic reaction and immunotherapy action depends on a different response of the immune system to the natural allergen (low exposure) compared to the allergen extract-vaccine (high exposure). MC: mast cell, EC: eosinophil, BC: basophil, DC: dendritic cell, BLC: B lymphocyte.

to gastrointestinal or skin symptoms, rhinoconjunctivitis. Importantly, there is currently no universally accepted grading system for local reactions following SLIT and this is urgently required in order to facilitate standardized reporting of local side effects. For example should GI symptoms be classified as local or systemic? One approach maybe to regard GI symptoms after SLIT as local, unless they occur in association with other systemic manifestations. No anaphylactic reactions have been reported in published trials, whereas 6 cases in 4 isolated case reports confirm that severe systemic reactions may very occasionally occur. However, all these case reports occurred in treatments given off license or in exceptional circumstances not conforming to recommended practice<sup>(89-92)</sup>.

### Comparison of SLIT with SCIT

There are very few adequately controlled head-to-head comparisons of SCIT vs SLIT whereas it is possible to observe the overall effect sizes of SCIT vs Placebo and SLIT vs Placebo in systematic reviews involving comparable patient groups. In this context, in a Cochrane systematic review of SCIT, where 51 publications met authors' inclusion criteria, symptom and medication score showed an overall reduction in the SCIT group (Standardized Mean Difference (SMD) -0,73 and -0,57, respectively) compared to the placebo group<sup>(93)</sup>. In a Cochrane systematic review of SLIT, including 60 R DB PC trials, significant reduction of symptom and medication scores were also found in the SLIT group (SMD -0,49 and -0,32 respectively) compared to the placebo group<sup>(94)</sup> (Table 1).

In a 3-year randomized placebo-controlled double-dummy

study that evaluated 71 adult birch hay-fever patients, the two administration routes were compared, in terms of efficacy and safety. Participants were treated for two consecutive years after a baseline year. No statistically significant difference was found between SCIT and SLIT, regarding symptom and medication scores, whereas the study was not adequately powered to detect a potential difference if one existed.

The current position is that the relative efficacy of SCIT vs SLIT is inconclusive and larger adequately powered head to head trials are needed before recommendations can be made for routine practice<sup>(95)</sup>. Meanwhile, both SCIT and SLIT have been shown to be effective in placebo-controlled trials and a major determinant of route of immunotherapy, SCIT or SLIT for seasonal pollinosis should be patient preference, since the indications are the same for both routes.

## MECHANISMS OF IMMUNOTHERAPY

### Allergic inflammation

Atopy refers to a genetic predisposition to develop IgE antibodies when exposed to common inhaled aeroallergens. The allergic reaction begins when an allergen reaches the skin or mucosal surfaces. Dendritic cells are the professional antigen presenting cells (APC) that capture allergen and process it internally into individual allergen peptides that are combined with and co-expressed on the cell surface with MHC Class II molecules. Allergen peptide recognition and specific binding by the T cell receptor, along with co-ligation of accessory molecules leads to T cell triggering and activation. Depending on the route of entry, nature of the allergen dose, APC and cytokine milieu,

Table 2. Systemic reactions to subcutaneous immunotherapy <sup>(76,77)</sup>.

	1981-1990	1991-2000
Systemic Reactions (N)	115	26
Patients (%)	5,2	1,08
Injections (%)	0,06	0,01

Table 3. Risk factors for systemic reactions after SCIT.

Exquisite sensitivity to allergen
Beta blockers
Unstable asthma
Rush protocols of SCIT
High doses of vaccines
History of previous systemic reactions
First dose from a new vial (and batch change)
Delay in the use of adrenaline
Dosing errors
Lack of cardio respiratory resuscitation facilities
Seasonal exacerbation of asthma symptoms

Table 4. Allergy clinic equipment required <sup>(84)</sup>.

Adrenaline 1:1000 for intramuscular use
Antihistamines and corticosteroids (peros, iv)
Short acting beta-sympathomimetic-agonist (MDI and Nebulizer)
Intravenous administration set, saline /colloids for IV infusion
Oxygen and suction equipment
Stethoscope, blood pressure and oxygen saturation monitoring devices
Peak flow meter
Tourniquet, masks, syringes, needles and intravenous cannulae

Table 5. Contra-indications for allergen immunotherapy.

Absolute	Relative
Serious chronic diseases	Pregnancy (SCIT initial phase)
Severe asthma (FEV1 <80%)	Severe atopic eczema
Treatment with beta-blockers	Lack of adherence

the interaction of the APC with the T cell leads to polarization of T lymphocytes into distinct subtypes. Low antigen concentrations at mucosal surfaces when B cells act as APCs favour the development of the so-called T helper 2(Th2) subset (Figure 1). Th2 cells produce the cytokines IL-4, IL-5, IL-9 and IL-13. IL-4 preferentially favours Th2 T cell development. IL-4 and IL-13 induce B cell heavy chain switching in favour of IgE. Recent data strongly support that IgE synthesis may occur locally in tissues in adequate amounts to initiate and maintain surface IgE-sensitisation of resident mast cells. IL-9 is a potent mast cell growth factor. IL-33 and IL-25, along with thymic stromal lymphocyte activation protein (TSLP) produced by the epithelium are considered important to maintain Th2 differentiation of T cells in tissues. During this early phase of sensitization, an army of T and B memory cells also develop <sup>(96)</sup> (Figure 1).

Re-exposure to the allergen leads to cross linking of adjacent IgE antibodies on the mast cell surface leading to degranulation and release of inflammatory mediators. These mediators are both membrane-derived (from arachidonic acid) including leukotrienes C4, D4 and E4, prostaglandin D2 and platelet-activating factor and granule-associated including histamine and tryptase. Their biological properties are consistent with inducing vasodilation, increased vascular permeability, neuronal activation and mucus production, consistent with the classic type 1 hypersensitivity symptoms of immediate itching, sneezing,

profuse nasal discharge, congestion, typical of hay-fever during seasonal pollen exposure <sup>(97)</sup>.

The late phase of an allergic reaction occurs 6 - 24 hours after allergen exposure and gives rise to principally nasal congestion and nasal sensitivity to nonallergic triggers that may persist for days or weeks. The late response is accompanied by the recruitment of other effector cells, like activated CD4<sup>+</sup> T cells, eosinophils, basophils and neutrophils, which infiltrate the tissues causing inflammation <sup>(98-102)</sup> (Figure 1).

### Specific allergen immunotherapy

SIT, in both subcutaneous and sublingual routes of administration, causes changes in T-cell and B-cell responses (Figure 2). The induction of T regulatory lymphocytes (T-regs) and especially their subsets, CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> Tregs and inducible Tr1 is crucial to achieve tolerance in SIT <sup>(103,104)</sup>.

The functions of Tregs involve both cell-cell contact and also the production of soluble factors such as the inhibitory cytokines IL-10 and TGF-β <sup>(105-107)</sup>. TGF-β upregulates the master switch gene FOXP3 in CD4<sup>+</sup>CD25<sup>-</sup> cells along with a change in phenotype to CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low</sup> cells (regulatory T cells) in parallel with their immunosuppressive capacity. TGF-β also drives IgA class-switching of B cells. IgA is a non inflammatory antibody and, like IgG4 is also produced following immunotherapy <sup>(108,109)</sup>. These critical early events involving regulatory T cells occur

within weeks of initiating immunotherapy. For example, IL-10 production by peripheral blood mononuclear cells occurred as early as two weeks at low allergen doses and paralleled suppression of the late cutaneous response<sup>(110)</sup>.

B cell tolerance is not as rapid at onset as T tolerance. It is associated with an increase in IgA2, IgG1 and particularly IgG4 antibodies that increase 10- to 100-fold during SIT<sup>(111,112)</sup>. IgG4 antibodies are capable of competing with IgE for allergen binding thereby preventing the formation of allergen-IgE complexes and subsequent cross-linking of IgE. In the context of FcER1-dependent events there is inhibition of mast cell and basophil activation, whereas the inhibition of allergen-IgE complex binding to B cells via FcERII (CD23) is a crucial factor in blocking IgE-facilitated antigen presentation and activation of Th2 cells<sup>(113,114)</sup>. In a 4-year study, patients sensitized to Grasses with moderate-to-severe allergic rhinitis underwent a randomized, double-blind, placebo-controlled discontinuation of SCIT. All subjects received SCIT for two years followed by a further two of either active or placebo injections. Clinical improvement was maintained after two years of discontinuation. Although immunotherapy-induced Grass pollen-specific IgG1 and IgG4 levels decreased by 80% during discontinuation, inhibitory bioactivity of allergen-specific IgG antibodies was maintained unchanged, implying persistence of lower quantities of IgG antibodies of high affinity and/or avidity that might contribute to persistence of long-term tolerance<sup>(115,116)</sup>.

An early effect of SIT is its ability to suppress allergen induced late phase reactions in the skin nose and lungs<sup>(117-119)</sup>. This effect seems to be associated with a significant decrease of the effector cells and consequently of the inflammatory response<sup>(117,120)</sup>. Eosinophil function and IL-5 production is downregulated by IL-10<sup>(121)</sup>. IL-10 also modulates the threshold of mast cell and basophil degranulation, thereby decreasing the release of inflammatory mediators<sup>(122)</sup>.

## BIOMARKERS OF CLINICAL RESPONSE TO IMMUNOTHERAPY

Most patients, if carefully selected according to immunotherapy guidelines<sup>(80,84)</sup>, demonstrate clinical improvement due to immunotherapy, while there is a minority who fail to respond. Ideally, changes in T-cell and B-cell responses could be used as biomarkers to predict immunotherapy success<sup>(123)</sup>.

Clinical trials of immunotherapy have revealed transient early increases in allergen-specific IgE that are followed by subsequent blunting of seasonal increases in IgE<sup>(124-126)</sup>. Probably these IgE antibodies are non-functional, presumably unable to effectively sensitize mast cells<sup>(123)</sup>. The calculation of the serum s-IgE/t-IgE ratio in patients monosensitized to Grasses, *Parietaria judaica*, *Olea europea*, and House dust mites showed significant correlation with clinical response to allergen specific immunotherapy<sup>(127)</sup>.

Increases in levels of allergen-specific IgG1 and IgG4 antibodies have been revealed in patients receiving immunotherapy<sup>(128,129)</sup>. However immunoreactive IgG levels have failed to correlate closely with the clinical response to treatment, whereas functional IgG-associated assays may be more predictive<sup>(130)</sup>.

IgE antibodies are captured on the B cell surface due expression of the low affinity IgE receptor CD23. Antigen presentation is facilitated by this process at lower allergen concentrations. The IgE-facilitated allergen binding assay (IgE-FAB) represents an in vitro model of facilitated allergen presentation and may be useful for monitoring IgG-associated serum inhibitory activity during allergen immunotherapy<sup>(131)</sup>. Additionally the functional role of IgG4 antibodies may be assessed by their ability to inhibit FcεRI-mediated basophil histamine release<sup>(110)</sup>.

IgA, like IgG4, is a noninflammatory immunoglobulin isotype. IgA2 levels correlated with increased local TGF-β expression, and induced IL-10 production from autologous monocytes. These data suggested that IgA antibodies, by augmenting IL-10 production, could contribute indirectly to the induction of tolerance in immunotherapy-treated patients<sup>(108)</sup>.

An alternative biomarker of efficacy of immunotherapy may be to measure expression of Th2 cytokines and allergic effector cells in nasal fluids. Thus Creticos showed a dose-dependent reduction in eosinophil numbers<sup>(132)</sup> and in inflammatory mediators<sup>(133)</sup> following immunotherapy. Recent studies have afforded the opportunity to measure tryptase, eosinophil cationic protein (ECP) and Th2 cytokines in minute quantities of nasal fluid collected on filter paper strips and/or nasal sponges<sup>(134)</sup> – whether these novel methods of detection of mediators and cytokines will predict responsiveness to immunotherapy remains to be tested.

These potential markers of successful immunotherapy require further evaluation in large randomized controlled studies.

## FUTURE

The efficacy and long term benefits of allergen Immunotherapy have provided incentive for other novel vaccine approaches with potential to retain/improve efficacy whilst improving safety and convenience for patients.

Allergoids are produced after chemical modification, for example, with glutaraldehyde or formaldehyde. Allergoids reduce IgE epitopes while preserving Tcell-epitopes. As a consequence allergoids might exhibit low allergenicity, with potential for less risk of IgE-mediated side effects. In a double-blind, placebo-controlled study with patients allergic to grass pollen, allergoid SCIT reduced symptom scores 27% after the first year and 48% after the second year compared to placebo treatment<sup>(135)</sup>.

The addition of Toll like receptor agonists, like TLR-4<sup>(136)</sup> and TLR-9<sup>(137)</sup> agonists, induce immune deviation in favour of Th1 responses in both murine models<sup>(138)</sup> and man<sup>(139)</sup> and reduce Th2 cytokine production. In a double-blind placebo-controlled

Table 6. Potential Biomarkers for monitoring allergen immunotherapy.

Allergen specific IgE <sup>(125, 126)</sup>
Specific IgE/total IgE ratio <sup>(127)</sup>
Allergen specific IgG1, IgG4 <sup>(128, 129)</sup>
IgE-FAB <sup>(131)</sup>
Allergen-specific IgA2 <sup>(108)</sup>
Local nasal eosinophils, mediators and cytokines <sup>(132 - 134)</sup>

study, 25 patients allergic to ragweed received six weekly injections of the TLR9 conjugated vaccine or placebo before the first ragweed season and were monitored during the next two ragweed seasons. The vaccine appeared to offer long-term clinical efficacy evident one year after a single pre-seasonal-injection protocol for ragweed allergic rhinitis <sup>(137)</sup>.

In addition to the sublingual route <sup>(69)</sup>, novel alternative routes include the epicutaneous route <sup>(140)</sup> and allergen injection directly into inguinal lymph nodes <sup>(141)</sup>.

Modern molecular biology has enabled the efficient cloning of many major allergens from the common allergen sources thereby allowing mass production of recombinant allergens for both diagnosis and therapy of allergic diseases. Recombinant vaccines include two types: wild type, which mimic natural allergen <sup>(111,142)</sup>, and genetically engineered hypoallergenic mutants <sup>(143)</sup>, which reduce allergenicity and increase immunogenicity <sup>(144)</sup>.

The use of small T cell peptide fragments similarly has been shown to decrease allergenicity <sup>(145,146)</sup>. There is a need for more studies of these approaches before their use can be recommended routinely in clinical practice.

Omalizumab is a humanized recombinant anti-IgE monoclonal antibody, approved for use in patients with moderate-to-severe perennial allergic asthma. The addition of anti-IgE before rush SCIT, as pre-treatment, reduced allergic reactions and provided good control of allergic rhinitis symptoms, with potential for enhanced and more prolonged inhibition of IgE-facilitated allergen presentation <sup>(147)</sup> according to a double-blind, parallel-group, placebo-controlled trial of ragweed sensitized patients <sup>(148)</sup>. Cost effectiveness and duration of omalizumab treatment remain issues to be resolved.

**CONCLUSIONS**

Specific allergen immunotherapy is highly effective in allergic rhinoconjunctivitis with/without mild controlled asthma. Subcutaneous immunotherapy has minimal risk when administered in a setting that permits recognition and instant treatment of rare systemic reactions. Choice of allergen(s) is based on detailed history, skin prick tests and specific IgE.

1. In clinical practice SIT can be administered via two routes

- subcutaneous (SCIT) and sublingual (SLIT).
- 2. SCIT is effective against many relevant allergens: pollens (grasses, trees, weeds), house dust mites, animal epithelia although little evidence of efficacy against moulds owing to lack of adequate standardized mould extracts availability. SLIT is highly effective against pollens, more studies are needed to confirm efficacy against perennial aeroallergens, particularly in children.
- 3. SIT alters the natural course of allergic respiratory disease, provides long-term remission and may reduce progression to asthma and onset of new sensitizations.
- 4. Patients allergic to one allergen are ideal for successful SIT although monotherapy SIT is equally effective in polysensitized patients provided their symptoms are largely explained by the allergen used for therapy. SIT with multiple different allergens in polysensitized patients is not recommended whereas use of more than one extract in patients with a limited spectrum of allergens is logical and justifies further evaluation.
- 5. Systemic reactions may rarely occur with the subcutaneous route. Trained experienced staff, adequate facilities for resuscitation and patient adherence are mandatory to avoid or treat these reactions.
- 6. Subcutaneous immunotherapy and more recent well-controlled large clinical trials of sublingual immunotherapy have been shown to be effective and well-tolerated also in children. In general, indications for immunotherapy are the same in children as for adults.
- 7. The mechanism of both subcutaneous and sublingual immunotherapy involves antigen-specific changes in T-cell and B-cell responses, the basis of altered immunological memory and long-term tolerance after immunotherapy is discontinued.
- 8. The induction during immunotherapy of T regulatory lymphocytes and especially their subsets, CD4 +CD25 high+CD127-FOXP3 Tregs and inducible Tr1 seems important to achieve tolerance, due to both the production of inhibitory cytokines, such as IL-10 and TGF-β, but also involving unknown mechanisms involving cell-cell contact. B cell alterations are evident as increases in IgA2, IgG1 and particularly IgG4 antibodies that compete with IgE and disrupt the formation of allergen-IgE complexes that bind to antigen-presenting cells and thereby inhibiting allergen presentation.
- 9. The discovery of biomarkers that are either surrogate and/or predictive of the clinical response to immunotherapy is likely based on a better understanding of the mechanism of immunotherapy.
- 10. Novel approaches to immunotherapy such as allergoids, Toll like receptor agonists as adjuvants, recombinant allergen vaccines and allergen-derived T cell peptides are currently under evaluation.



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## Conflict of interest

The authors have no conflict of interest to disclose. The authors have no conflict of interest to disclose.

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