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REVIEW

Allergen-specific immunotherapy in allergic rhinitis and asthma. Mechanisms and proof of efficacy

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Summary

Appeared at the beginning of the 20th century, allergen-specific immunotherapy (SIT) has long been used in allergic rhinitis and asthma without any knowledge of its mechanisms of action or any tangible proof of its efficacy. However, from the beginning of the era of evidence-based medicine, a number of placebo-controlled studies have been published and reached a sufficient number to assess the cellular events induced by SIT and allow meta-analysis to provide guidelines based on proofs. Controlled studies and meta-analysis concerned not only subcutaneous immunotherapy but also the sublingual route, demonstrating an effect of SIT on symptoms and medication use. Most recently sublingual tablets were proposed in allergic rhinitis. This paper reviews the mechanisms of SIT, the evidence of efficacy of SIT from the injective to the sublingual route and reminds the current guidelines.

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Introduction

Together with allergen avoidance, allergen-specific immunotherapy (SIT) is the only available treatment able to affect the natural course of allergy. It is validated for IgE-mediated sting allergies and respiratory allergies, i.e. allergic rhinitis and asthma. Since the first use of SIT in the early 20th century,¹ a large amount of clinical trials have been conducted and published, most of them being placebo-controlled. These trials made SIT become an evidence-based treatment and brought insights into SIT mechanisms, efficacy and safety. These insights served to figure out indications and contra-indications of SIT, and the necessary rules to be applied to improve the benefit/risk ratio of this treatment. These rules were relayed in international guidelines. More recently, allergen extracts were successfully proposed as sublingual tablets in allergic rhinitis. In this review, we will review the mechanisms of SIT, go through the main published clinical data concerning SIT in allergic respiratory diseases, insist on most recent papers, and remind the current guidelines.

Allergen-specific immunotherapy: a model of immunomodulation in humans

Prevention of inflammation in response to non-dangerous antigens has long been a major issue in clinical immunology, with a variable success in organ transplantation, autoimmune and allergic diseases through the use of controller drugs such as corticosteroids, calcineurin inhibitors and more recently monoclonal antibodies. However, these drugs are not antigen specific and therefore induce a state of immunosuppression. They also induce some adverse non-immunological effects. In the field of allergy, however, antigen-specific immunotherapy drives the immune system to tolerate allergens without controllers in the long term. This antigen-specific immunomodulation is a unique and inestimable tool to understand not only the treatment, but also the disease it cures.

Allergy is currently considered as a peculiar case of an inflammatory reaction, in which the antigen is an allergen, and the host an allergic subject. Because it is an allergen

penetrating into an allergic organism, the antigen presentation results in a Th2 differentiation of specific T cells (Fig. 1). Schematically Th2 cells, by producing IL-4 and IL-13, trigger the IgE synthesis, while by producing IL-5 they attract and activate eosinophil polymorphonuclear cells. Then IgEs bind their high affinity receptor (FcεRI) on effector cells such as mast cells and in case of continuous exposure or re-exposure induce mast cell degranulation, histamine and leukotriene release and the early phase of the allergic reaction. In parallel, in the presence of allergens, IgEs bind to B cells and other antigen presenting cells through the low affinity receptor of IgE, CD23. Eosinophils, through their production of basic proteins, injure epithelia, organize the late phase response and the chronic allergic reaction. SIT acts at each step of the allergic reaction: IgE and IgG productions, mast cell and eosinophil homing, T cell activation, and antigen presentation.

Action of SIT on Ig production

The elevation of specific IgG during SIT, notably IgG4, was described a long time ago,^{2,3} and confirmed more recently.⁴ Although they can be involved in allergic reactions, IgG4 are widely considered as blocking antibodies, preventing the allergen to encounter IgE bound on FcεRI at the surface of effector cells and antigen presenting cells. The blocking antibodies also prevent the binding of allergen–IgE complexes on the low affinity receptor for IgE at the surface of B cells, thus decreasing the capacity of B cells to present the allergen to specific T cells. Indeed, a dramatic decrease under SIT vs. placebo of the IgE binding to B cells in grass pollen allergy was shown.⁵ This action of blocking antibodies was also shown in birch allergy.⁶ Most recently, a parallelism between the inhibition of early skin response, histamine release, IgG binding to B cells and IgG4 increase was demonstrated.⁷ Another hypothesis for the action of IgG4 during SIT is the preferential engagement of the B isoform of the low affinity receptor to IgG (FcγRIIB) by IgG4–allergen complexes on mast cells that would induce a deactivation signal through phosphorylation of immunoreceptor-based inhibition motifs (ITIM) activating intracellular phosphatases, counterbalancing the effect of immunoreceptor-based activation motifs (ITAMs) present in the intracellular tail of FcεRIγ.⁸

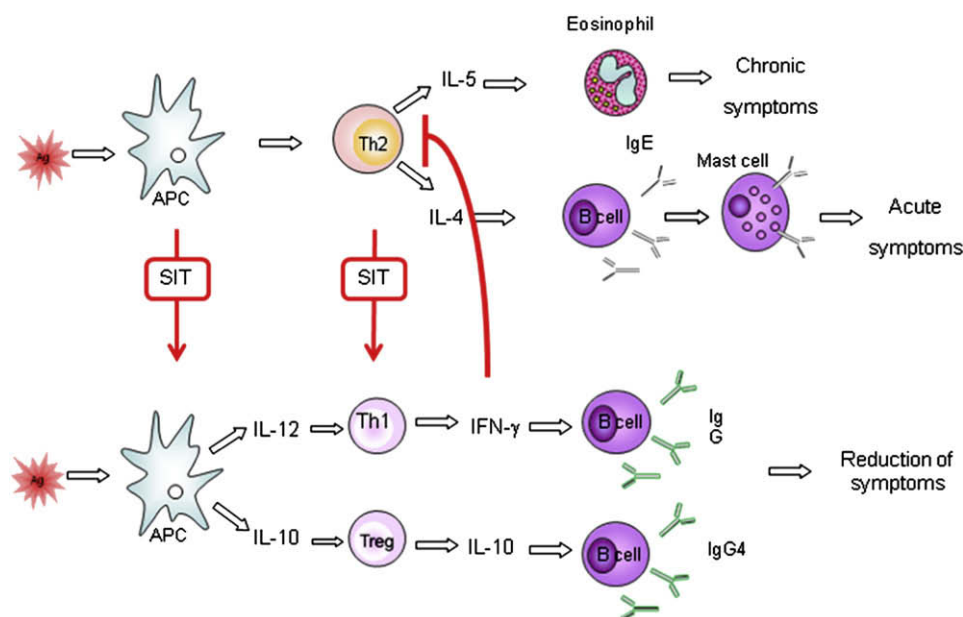


Figure 1 Antigen presentation results in a Th2 differentiation in allergy, leading to the eosinophil and mast cell mediated inflammatory reaction. Specific immunotherapy (SIT) acts both on antigen presenting cells and T cells to prevent symptoms. Ag: antigen, APC: antigen presenting cell.

Action of SIT on eosinophil and mast cell homing

A decrease in tissue infiltration by eosinophils was shown during sublingual immunotherapy to house dust mites.⁹ In this study, the conjunctival infiltration by eosinophils was decreased after challenge by allergens in the treated group but not in the placebo group. Importantly after 2 years of treatment, the lower infiltration by eosinophils was found before challenge in the treated group compared to placebo. During subcutaneous SIT against grass pollen allergy, an inhibition of eosinophil migration into the nasal mucosa after challenge¹⁰ was demonstrated by a decrease in intra-epithelial eosinophil infiltrate.

With regard to mast cells, apart the effects related to the IgG4/IgE increase, a decrease of the allergen-induced c-kit positive cell infiltration of the nasal mucosa has been shown after grass pollen SIT compared to placebo.¹¹

Action of SIT on T cell activation

It is in the field of T cell activation that the main studies on SIT mechanisms have been published in the last decade. After the discovery of the Th2 model in the early 1990s, it was postulated that SIT was able to trigger a Th2 to Th1 switching of T cell activation, thus inhibiting eosinophils, mast cells and IgE production and drive the isotypic commutation towards IgG4. Indeed, Secrist demonstrated in blood that allergen stimulation was inducing IL-4 production in non-treated patients and IFN- γ in patients desensitized to house dust mites or grass pollen.¹² Accordingly, an increase of IFN- γ producing T cells was observed upon pollen challenge in treated rhinitic patients.¹³

Hymenoptera venom allergy provided a paradigm for the study of T cell activation during SIT. Indeed hymenoptera

venom allergic patients selected for immunotherapy constitute a homogeneous group of patients, all having experienced a generalized anaphylactic reaction after a unique allergen challenge. In addition, as being usually non-atopic, these patients' immune system do not respond to other allergens during treatment, and thus SIT effects on T cells are easier to detect. Lastly, SIT is considered as regularly efficient in these patients, which strengthen the reliability of this experimental model. The Th2 to Th1 shift was first reported in venom SIT.¹⁴ However, in bee venom allergy, the ex-vivo stimulation of T cells by PLA2, the main allergen in bee venom, induced an extinction of both Th1 and Th2 cytokine release in treated patients. By contrast, a dramatic increase of IL-10 production was observed during SIT.^{5,15} IL-10 is a potent immunosuppressive cytokine, well known to be involved in tolerance induction and maintenance. It was, however, only in the early 2000s that T regulatory cells (Treg) were identified as IL-10 producers during SIT. Treg cells represent small populations of T cells, able to produce IL-10 and/or TGF- β , another immunosuppressive cytokine. Among Treg cells, the natural subset, expressing CD4 and CD25 at a high level together with the transcription factor fox p 3, is the most studied to date. Several papers were published within the last years about Treg cells in allergy and other inflammatory diseases, pointing out a Treg deficiency as responsible for many of them. It is probably excessive to consider the Treg cell impairment as the *primum movens* of allergy in all patients, as it can also reflect the consequence of inflammation and as the relevance of fox p 3 detection as a proof of their regulatory phenotype is debated.¹⁶ Nevertheless, CD4+CD25+ cells were shown to be decreased and ineffective in grass¹⁷ and birch pollen and house dust mite allergies.¹⁸ During SIT against grass pollen¹⁹ and venom,²⁰ an increase in Treg cell activation was shown. We

demonstrated a progressive increase of the proportion of CD4 + CD25 + high T cells and of IL-10 producing T cells during venom SIT, paralleled with a Th2 to Th1 shift. This effect of SIT was detectable as soon as 6 h after the first SIT injection. Importantly, results differed between patients having experienced severe vs. non-severe reactions, with an early increase of Treg cells in the latter, associated to a clearer IL-10- and IFN- γ -positive cells ascension in this group.²¹ Most recently, we showed that in bee venom SIT, adverse anaphylactic reactions induced by the treatment itself were related to an absence of Treg and IL-10+ cell induction, and of the Th2 to Th1 switch, making this phenomenon a potential tool to set up SIT progression (Botturi, in preparation). Indeed in patients with an absence of early Treg increase upon SIT, intermediate doses could be administered during the treatment to prevent adverse events. Interestingly, such strategy can be proposed because of a very early modification of T cell activation during SIT (after 4 h), which contrasts with the modification in Ig concentrations that are not related to the clinical benefit.

In grass pollen allergy, an increase of IL-10 positive cells infiltrating the nasal mucosa of challenged patients after SIT was shown vs. placebo.⁵ Interestingly the IL-10 increase paralleled the skin late phase response inhibition.⁷ Completing the picture, Fox p 3 + CD4 + CD25 + T cells were detected in the nasal mucosa after SIT,²² as well as TGF-beta+ cells.²³ It is noteworthy that SIT-induced IL-10 production is not limited to T cells. Indeed it was well demonstrated in B cells and monocytes during bee venom allergy,¹⁵ and in macrophages during seasonal rhinitis.⁵

A few studies concerned the mechanisms of sublingual immunotherapy, and the recent publications suggest that the mechanisms involved are similar as those involved in the injective route. In birch allergy, Bohle and colleagues detected an increase of circulating CD4 + CD25 + cells, of IL-10 and fox p 3 gene expression as early as 4 weeks after the onset of sublingual SIT.²⁴

Concerning the SIT action on T cell activation, it must be said that¹ the induction of Treg cells was probably detected as early as 1980 in a paper demonstrating the presence of a cell population in desensitized patients inhibiting the proliferation of ragweed specific T cells in desensitized patients only,²⁵ and² that almost all the literature concerns the injective route.

Action of SIT on innate immunity

Above T cells, SIT acts on antigen presenting cells themselves. This is of matter since this action *de facto* proves a non-allergen-specific role of SIT. This non-specific action could be suspected by considering the number of T cells involved in the Treg increase and/or the Th2 to Th1 shift. Indeed this number is too high to reflect allergen-specific T cells only, and necessarily bystander non-specific T cells are involved. This possible implication of bystander T cells²⁶ could be relevant to the known effect of SIT on the natural history of allergy, notably new sensitizations.²⁷

SIT is administered as purified and standardized extracts that contain allergens accompanied by non-protein components able to bind to toll like receptors (TLR) present

at the surfaces of professional antigen presenting cells (APCs) and non-professional APCs (epithelial cells, B cells, endothelial cells, etc). These motifs called pathogen-associated molecular patterns (PAMPs) include small sequences of nucleic acids such as single or double strand RNAs, CpGs, or saccharides or lipopolysaccharides. The binding of TLR by PAMPs induces APC to differentiate into cells inducing one or another kind of T cell differentiation independently of the nature of the antigen itself.²⁸ It is likely that during SIT, PAMPs associated to allergens but also to other proteins present in the extract orientate APC in a pro-Th1 or a pro-Treg profile, thus participating to the immune effect induced by SIT. It is also possible that because of the route, rhythm and dose of allergen administered, the APC behaves in a different manner than it does when the exposure is natural, and therefore orientates T cells to a tolerant pathway. Beyond these speculations, several studies have demonstrated an effect of SIT on innate immunity. During venom SIT, Akoum showed that the SIT-induced decrease of IL-4 by T cells was seen only after ex-vivo stimulation by the allergen, whereas the increase in IFN- γ production was visible without ex-vivo stimulation.²⁹ In this model, we reported an increase of IL-12, a pro-Th1 cytokine, by blood monocytes.³⁰ In the nasal mucosa, SIT induces IL-12 producing cells.³¹ This effect of SIT on innate immunity is probably important, and questions the relevance of using recombinant allergens for immunotherapy, unless they are associated to non-specific adjuvants such as PAMPs.³² The effect of SIT on cells belonging to the innate immunity is not limited to IL-12 production. Indeed several works clearly demonstrated that monocytes and macrophages were producing IL-10 in treated patients.^{5,15}

With regard to the sublingual route, a recent study showed that a TLR-2 agonist able to trigger the IL-10 and IL-12 productions by dendritic cells inducing in turn T cell-IL-10 and IFN- γ productions was able to enhance the effect of sublingual ovalbumin in a model of ovalbumin-induced asthma.³³ A similar strategy was already used in humans by coupling a TLR 9 agonist, i.e. CpG sequences, to Amb A 1, a recombinant form of the major allergen of ragweed. A prolonged effect on rhinitic symptoms was obtained with the use of a weekly injection during pollen season.³¹

Allergen-specific immunotherapy: an evidence-based treatment of allergy

The efficacy of SIT has long been a matter of debate.^{34,35} Indeed, it has been considered as an adjunctive treatment with poor efficacy as long as controlled trials were not available. Furthermore, as SIT is not effective for all the allergens used, nor in all the patients treated, it has been very difficult, despite its ancient availability, to convince the scientific community of its efficacy. Lastly, it must be said that it is especially difficult to demonstrate an effect for allergen-specific immunotherapy. Indeed, in allergic diseases, placebo effect is always consistent, especially when the treatment consists of repeated injections. The fact that increasing doses of a vaccine are injected adds some irrational beliefs in the efficacy, increasing the placebo effect. Furthermore, it is especially difficult to embark patients in a long-term trial against placebo, using

the injective route. Such trials are particularly difficult to set up for children in whom they are felt as unacceptable. However, since a couple of decades, a large work of analysis of old trials and of setting of new trials has been done, giving to SIT its nobility and making it considered as an evidence-based treatment of allergy.

Efficacy of subcutaneous immunotherapy (SCIT) in allergic rhinitis

Allergic rhinitis is certainly the disease in which SCIT efficacy is the most documented and proved. A meta-analysis was published in 2007 in the frame of the Cochrane collaboration.³⁶ Fifty-one trials including a total of 2871 participants were considered. None of them was conducted exclusively in children, but participants younger than 18 years of age were included in 9 studies. Allergens tested were ragweed ($n = 12$), mixed grass ($n = 16$), timothy ($n = 5$), parietaria ($n = 6$), birch ($n = 4$), orchard ($n = 2$), cedar ($n = 3$), bermuda ($n = 1$), juniperus ashei ($n = 1$) and cocos ($n = 1$). Fifteen studies suitable for symptom score analysis demonstrated a significant reduction (standardized mean difference (SMD): -0.73 , 95% CI: -0.97 to -0.50 , $p < 0.00001$). The medication score analysis (13 studies) showed a significant reduction in the SIT group (SMD: -0.57 , 95% CI: -0.82 to -0.33 , $p < 0.00001$). It is of note that there was a significant heterogeneity among these studies. Passalacqua and colleagues³⁷ updated this work in the frame of the GA²LEN network by collecting 15 recent studies published between 2000 and 2006. Reduction of symptoms and/or need for medications were confirmed with grass, birch, parietaria and ragweed pollens, and house dust mites (HDM). In addition, SCIT treated patients' quality of life significantly improved.^{38–40}

It is for allergic rhinitis that the duration of SCIT effect is the best documented. In 1988 Mosbech and colleagues⁴¹ had reviewed after 6 years of discontinuation of SCIT for grass pollen allergy 38 patients treated initially for 2.5 years. Symptom scores had remained unchanged at review. A cohort of 22 patients with previous SCIT was reviewed 12 years after discontinuation of therapy.⁴² Again, symptom and medication scores were still better in treated patients compared to controls. Des Roches and colleagues⁴³ followed-up for 3 years every 6 months a cohort of 40 patients desensitized for a period of 12–96 months. Fifty per cent of patients had relapsed after 1 year and 60% at the end of the 3 years of follow-up. The authors showed that the shorter was the treatment, the earlier was the relapse. Naclerio and colleagues conducted a 3-year controlled study in 20 patients.⁴⁴ After 3 years of ragweed immunotherapy, patients were randomized to receive either maintenance injections or placebo. Nasal challenges had been performed before treatment, and repeated before and 1 year after randomization. At the end of the 3 years course of SIT, symptoms had decreased as well as nasal challenge responses. One year after randomization, nasal challenges showed partial recrudescence in patients under placebo and remained inhibited in patients still treated. However, symptoms did not relapse in any group during the pollen season. Lastly, Durham and colleagues conducted a 3-year

study in which three groups of grass allergic patients were followed-up as to their symptom and medication scores among three successive pollen seasons.⁴⁵ In two groups (32 patients), SCIT had already been administered since 2 years, the last being a non-desensitized control group (15 patients). In the first treatment group, SCIT was maintained for 3 additional years whereas in the second it was discontinued and replaced by placebo. In both immunotherapy groups, scores did not vary throughout the three seasons, remaining significantly decreased compared to the non-desensitized group.

Patients included in the PAT study,⁴⁶ a 3-year randomized, placebo-controlled trial, conducted in 205 grass and/or birch pollen allergic children, were followed after the end of the study.⁴⁷ A persistent clinical effect on conjunctivitis and rhinitis, evaluated on Visual Analogue Scale, was demonstrated 7 years after the study.

All these studies therefore clearly indicate a remnant effect of SIT after discontinuation of the treatment.

Efficacy of SCIT in allergic asthma

The proof of SIT efficiency in asthma has long been served by a meta-analysis by Abramson and colleagues, first published in 1995,⁴⁸ and then kept regularly up to date in the frame of the Cochrane Institute.^{49–51} The last update includes 75 controlled trials (23 studies added since the previous update), among which a large majority is double blind, placebo-controlled. These studies concern house dust mites ($n = 36$), pollen ($n = 20$), animal dander ($n = 10$), cladosporium ($n = 1$), latex ($n = 1$), and 6 further studies were using multiple allergens. This meta-analysis shows that globally 4 patients have to be treated by SIT to prevent one asthma attack and that 5 patients must be treated to prevent an increase of symptoms. Furthermore, it demonstrates that SIT in asthma results in a reduction of related medications, with a standardized mean difference (SMD) of -0.8 (95% CI: -1.13 to -0.48). Although asthma symptom and medication scores improved, the meta-analysis still failed to demonstrate a statistically significant effect of SIT on lung function, probably due to the heterogeneity of parameters measured, and to the limited size of the effect. A modest but significant effect of SIT (SMD: -0.43 , 95% CI: -0.71 to -0.14) on non-specific bronchial hyperreactivity was shown. With regard to specific bronchial hyperreactivity, the effect was more consistent (SMD: -0.66 , 95% CI: -0.87 to -0.45), and was the highest for house dust mites. Concerning immunotherapy with multiple allergens in polysensitized patients, the results were negative.

Some recent well-conducted studies do not figure the meta-analysis but confirm the data. Three studies, two in adults^{52,53} and one in children⁵⁴ were conducted in asthmatics allergic to house dust mite, and demonstrated a high efficacy on symptom and medication scores, and also on bronchial hyperreactivity. In birch pollen allergy, a 2-year study confirmed the efficacy of this treatment,⁵⁵ with again a significant decrease of clinical scores. In children with grass pollen allergy and seasonal asthma, seasonal SIT improved symptom scores and specific bronchial, conjunctival and cutaneous reactivity.⁵⁶

Thus, the proofs of subcutaneous SIT efficacy in asthma are built on at least 80 clinical trials. Again it is likely that some negative studies were not published, but the first version of Abramson's meta-analysis estimated already at 33 the number of studies necessary to inverse the conclusions driven.⁴⁸

Efficacy of sublingual immunotherapy (SLIT)

Because of the constraint due to the injective route, many efforts were done during the last 15 years to develop some oral immunotherapy. Most recent SIT studies concerned the sublingual route and were conducted throughout the last 10 years. To date, SLIT has been tested in rhinitis and asthma in about 40 double blind, placebo-controlled studies (sources: MEDLINE database). As most of these studies were included in the meta-analyses described below, they will not be described in detail. Indeed, 4 meta-analyses were published in the frame of the Cochrane collaboration⁵⁷ or with the Cochrane collaboration method^{58–60} (Tables 1 and 2). For all these meta-analyses the methodology consists of systematically reviewing the main databases including at least MEDLINE, EMBASE and the Cochrane Library, and then select double blind, placebo-controlled trials. Some meta-analyses also include open studies. Wilson and colleagues⁵⁷ considered 22 trials (979 patients) mixing adults and children with allergic rhinitis and/or asthma in a meta-analysis where pollen (grass: $n = 5$, parietaria: $n = 5$, olive tree: $n = 2$, ragweed: $n = 1$, tree: $n = 1$, cypress: $n = 1$) more than indoor allergen extracts (house dust mites: $n = 6$, cat: $n = 1$) were used. Despite the heterogeneity within studies in the age of the enrolled patients, the allergens employed, the scoring systems used and the durations of treatment chosen, conclusions could be driven: a significant reduction of symptom score and the primary outcome in all the studies included (SMD: -0.42 , 95% CI: -0.69 to -0.15 , $p = 0.002$) was demonstrated, along with a marked reduction of medication scores (SMD: -0.43 , 95% CI: -0.63 to -0.23 , $p = 0.00003$). Importantly, subgroup analysis failed to show a significant effect of house dust mite extracts (studies = 6, subjects = 118, SMD: -0.58 , 95% CI: -1.43 to 0.27 , $p = 0.18$). SLIT to cat could not be analysed because of a single trial meeting the required criteria to enter the meta-analysis. When considering separately adults and

children, the results were positive for adults ($n = 16$, subjects = 741, SMD: -0.4 , 95% CI: -0.61 to -0.18 , $p = 0.0003$), but clearly negative for children ($n = 5$, subjects = 218, SMD: -0.31 , 95% CI: -1.32 to 0.7 , $p = 0.5$). However, the small number of studies and subjects in each subgroup and their heterogeneity incite to consider negative results cautiously. No conclusion was driven about asthma alone.

Another meta-analysis focused on rhinitis in children.⁵⁸ Among 70 articles reviewed, only 10 fulfilled the selection criteria (total 577 patients, 484 evaluable), and were included. Again, the significant heterogeneity among studies should be noticed. Here, a significant reduction of both symptoms and medication use was found (respectively, SMD: -0.56 , 95% CI: -1.01 to -0.10 , $p = 0.02$, and SMD: -0.76 , 95% CI: -1.46 to -0.06 , $p = 0.03$). The higher number of children included (484 against 218), and the paediatric specificity of this study probably accounts for this difference with the Wilson's study. However, the low magnitude of the effect observed must be considered.

The sub-analysis according to the allergens used confirmed the significant effect to be due to pollen and not house dust mite extracts.

A recent additional meta-analysis in children with allergic asthma including 9 studies in 441 children allergic to house dust mites (6 studies) or pollen (3 studies),⁵⁹ concluded to an effect of SLIT on both symptom score (SMD: -1.14 , 95% CI: -2.1 to -0.18) and medication score (SMD: -1.63 , 95% CI: -2.83 to -0.44) (Table 1). No subgroup analysis according to the allergens used was done, but the authors indicated that reduction in asthma symptoms was constant in 5 of the evaluated studies, "particularly in those where mite extracts were used".

A last meta-analysis focused on asthma.⁶⁰ Twenty-five studies were retained, with 6 open clinical trials. A significant improvement in asthmatic symptoms considered as categorical outcomes was observed in 7 studies (876 patients, RR = 0.48). Interestingly, 4 studies (144 patients) showed a significant improvement in FEV₁, and FEV₂ studies an improvement in FEF_{25–75}. However, the effect on asthma symptoms considered as continuous outcomes and medication score were not significant (SMD: -0.38 , 95% CI: -0.79 to 0.03 ; SMD: -0.91 , 95% CI: -1.94 to 0.12 , respectively).

Table 1 Published meta-analyses regarding SLIT and SCIT.

	Abramson et al. (2003) ⁵¹	Calderon et al. (2007) ⁷⁵	Wilson et al. (2005) ⁵⁷	Penagos et al. (2006) ⁵⁸	Calamita et al. (2006) ⁶⁰	Penagos et al. (2008) ⁵⁹
Route	SCIT	SCIT	SLIT	SLIT	SLIT	SLIT
Disease	Asthma	Rhinitis	Rhinitis	Rhinitis	Asthma	Asthma
N studies	75	51	22	10	25	9
N patients	3506	2871	979	577	1706	441
Age	Adults + children	Adults + children	Adults + children	Children	Adults + children	Children
Symptom score SMD (95% CI)	-0.72 (-0.99 -0.33)	-0.73 (-0.97 to -0.50) $p < 0.00001$	-0.42 (-0.69 -0.15) $p = 0.002$	-0.56 (-1.01 -0.1) $p = 0.02$	-0.38 (-0.79 0.03) NS	-1.14 (-2.1 -0.18) $p = 0.02$
Medication Score SMD (95% CI)	-0.8 (-1.13 to 0.48)	-0.57 (-0.82 -0.33) $p < 0.00001$	-0.43 (-0.63 -0.23) $p = 0.00003$	-0.6 (-1.46 -0.06) $p = 0.03$	-0.91 (-1.94 0.12) NS	-1.63 (-2.83 -0.44) $p = 0.007$

SMD = standardized mean difference.

Table 2 Heterogeneity between studies about SLIT.

	Wilson et al. (2005) ⁵⁷	Penagos et al. (2006) ⁵⁸	Calamita et al. (2006) ⁶⁰	Penagos et al. (2008) ⁵⁹
Population	Adults + children	Adults + children	Children 3–18 years	Children 3–18 years
Years of publication	1966–2002	1966–2005	1966–2006	1966–2006
Allergens	House dust mites ⁽ⁿ⁼⁶⁾ Grass pollen ⁽ⁿ⁼⁵⁾ <i>Parietaria</i> ⁽ⁿ⁼⁵⁾ Olive ⁽ⁿ⁼²⁾ Ragweed ⁽ⁿ⁼¹⁾ Cat ⁽ⁿ⁼¹⁾ Tree ⁽ⁿ⁼¹⁾ Cupressus ⁽ⁿ⁼¹⁾	Pollen ⁽ⁿ⁼¹⁴⁾ House dust mites ⁽ⁿ⁼⁸⁾ Mix of allergens ⁽ⁿ⁼²⁾ Latex ⁽ⁿ⁼¹⁾	Grass pollen ⁽ⁿ⁼⁴⁾ House dust mites ⁽ⁿ⁼⁴⁾ <i>Parietaria</i> ⁽ⁿ⁼¹⁾ Olive ⁽ⁿ⁼¹⁾	House dust mites ⁽ⁿ⁼⁶⁾ Grass mix ⁽ⁿ⁼¹⁾ <i>Olea europaea</i> ⁽ⁿ⁼¹⁾ Pollen mix ⁽ⁿ⁼¹⁾
Symptoms considered	Rhinitis	Asthma±Rhinitis	Rhinitis	Asthma
Duration of studies	2–24 months	3 months–3 years	3–36 months	3–32 months

These meta-analyses therefore show SLIT to be efficient in pollen-induced rhinitis and asthma, both in adults and children, but indicate that more investigations must be provided to prove the efficacy of SLIT in house dust mite allergy. In addition it is noteworthy that¹ in some pollen studies, positive results were delayed to the second year of treatment^{61,62} and ²studies using a mix of various pollen extract are negative,⁶³ or marginally positive.⁶⁴

Some additional recent studies were not included yet in meta-analyses. One recent double blind, placebo-controlled study is available with cat standardized extracts.⁶⁵ The main outcomes were symptom scores and peak expiratory flow (PEF) during exposure to a cat in a cat-room, after 1 year of treatment. All patients suffered from rhinoconjunctivitis, and 75% from asthma. Symptoms and PEF significantly improved in the treatment group. It is noteworthy that cat exposure in a cat-room cannot be considered as reflecting a usual daily exposure, but this study is the first controlled one showing a significant clinical effect of a sublingual extract for cat allergy. The precedent study by Nelson et al.⁶⁶ was negative, but methodological problems have been pointed up.

The studies reported above were mainly conducted with sublingual drops of extracts. Recently, sublingual orodispersible allergen tablets were developed in grass pollen immunotherapy, and are expected to rapidly replace the liquid formulation. An orodispersible grass allergen tablet 75,000 standardized quality tablet (75,000 SQ-T), containing *Phleum pratense* allergens (approximately 15 µg of major allergen Phl p5) was tested in 11 studies. These allergens were selected for their wide expression in grasses.⁶⁷ An international work by Laffer and colleagues,⁶⁸ including 183 patients allergic to grass pollen from different populations (Europe, Canada, Japan), showed that recombinant allergens such as Phl p5 and Phl p1, bound high levels of timothy grass pollen-specific IgE in all subgroups.

The main originality of the tablet approach is the administration of a single pollen at a single dose, without any dose progression. Despite the immediate administration of a maximal dose of allergen, safety studies were convincing in adults,^{69–71} both in rhinoconjunctivitis and asthma, and more recently in 5–12 years old children⁷² with a limited number of benign allergic adverse events. A total of 634 adults from 51 centers in 8 countries were

included in a double blind placebo-controlled study to judge the clinical value of 75,000 SQ-T.⁷³ There was a significant improvement in rhinoconjunctivitis symptom and medication scores (reduction of 30% and 38%, respectively, $p < 0.0001$ for both). In another study, the quality of life appreciated with the validated RQLQ questionnaire was improved by 75,000 SQ-T.⁷⁴ Interestingly, this effect was higher than this of loratadine. Calderon and colleagues analysed the results of 3 positive controlled studies and suggested that a longer preseasonal treatment (8 weeks vs. 4 weeks) improved the clinical efficiency.⁷⁵ Although 75,000 SQ-T is a single pollen extract administered at a constant dose, tablets of immunotherapy are also developed as a mixture of equal proportions of 5 grass pollens (orchard, meadow, perennial eye, sweet vernal, and timothy grass) administered at a dose of 300 IR (approximately 25 µg/ml of the group 5 major allergens) reached after 5 days of titration.⁷⁶ In an international randomized, placebo-controlled double blind study, this tablet immunotherapy reduced the total rhinoconjunctivitis symptom score by 35% ($p = 0.0006$) in the 500-IR group and 37% in the 300-IR group ($p = 0.0001$). Individual symptom scores, rescue medication usage, and quality of life were also significantly improved by treatment. A low dose of 100-IR was inefficient.⁷⁶ Although these studies with sublingual tablets of grass pollen are promising, two studies in house dust mite allergy provided conflicting results. In the study by Passalacqua and colleagues,⁷⁷ among rhinitic adults, the composite asthma and rhinitis clinical score was improved the first but not the second year of treatment, whereas the rhinitic symptoms were still improved during the second year. The medication score was improved only at the first year.⁷⁷ The study by Pham-Thi and colleagues was clearly clinically negative in asthmatic children,⁷⁸ although skin sensitivity to house dust mite extracts significantly reduced and specific IgE and IgG4 significantly increased.

Only a few studies compared the sublingual to the injective route of SIT^{79–82} (Table 3). None of them detected a significant difference between both. However, two were not placebo-controlled,^{79,81} which renders difficult the interpretation of the results. Kinchi and colleagues performed a 2-year study in birch pollen allergic rhinitis. Forty-eight patients were randomized in three groups: sublingual tablets, subcutaneous depot extracts and placebo.⁸⁰ Again

Table 3 Main studies comparing SCIT to SLIT.

	Quirino et al. (1996) ⁷⁹	Mungan et al. (1999) ⁸²	Khinchi et al. (2004) ⁸⁰	Mauroa et al. (2007) ⁸¹
Method	Double blind (double-dummy), but not placebo-controlled	Randomized, placebo-controlled	Randomized, double blind, double-dummy, placebo-controlled	Randomized but not placebo-controlled
Disease	Rhinitis	Rhinitis and asthma	R±A	R
Allergen	Grass	House dust mites	Birch	Betulaceae
Ratio SL/SC dose	2.4	~100	~175	~100
N patients	20	36	71/48	47/40
Age	Adults	Adults	Adults	Adults
Duration	Two pollen seasons	1 year	2 years	One pollen season
Medication score	SLIT/SCIT: NS	SLIT/placebo: $p < 0.01$ SCIT/placebo: $p < 0.01$	NS between SC and SL SL/placebo: $p < 0.02$ SC/placebo: $p < 0.002$ (first season)	NS between SC and SL
Main symptom score	SLIT/SCIT: NS	SLIT/placebo $p < 0.01$ for rhinitis but NS for asthma SCIT/placebo: $p < 0.01$	SL/placebo: $p < 0.002$ SC/placebo: $p < 0.002$ (first season)	
Immunologic evaluation	Only in the SC group: change in IgG4 ($p < 0.001$)	SLIT/SCIT: NS Increase of IgG4 in both SLIT and SCIT	No data	Only in the SC group: significant increase in IgG4 ($p = 0.01$)
Safety: systemic reactions	No data	None	29% in the SC group 0% in the SL group	16% in the SC group 0% in the SL group

NS = not significant.

this study failed to show a difference between both routes of SIT. Both treatments improved significantly the symptom score compared to placebo during the first year. However, only subcutaneous SIT was efficient on medication score. During the second season, pollen counts were reduced so that no conclusion could be driven.

With regard to adherence to treatment, the advantage of an administration route to another is not established yet. Some studies found a very high rate of adherence to SLIT, between 75 and 100%.^{37,83} In contrast, Pajno and colleagues in a 3-year study,⁸⁴ found adherence to the subcutaneous-route to be higher than this to SLIT, with 10.9% of non-adherent patients in the former and 21.5% in the latter. Non-compliance was defined by stopping SIT without the authorization of the prescribing physician.

Does immunotherapy modify the natural course of allergy?

In addition to its effect on symptom and medication scores, some studies have showed that immunotherapy was able to prevent from further sensitizations and to reduce the risk of asthma in rhinitic subjects. Indeed in a paediatric study in asthma, Des Roches, during a 3-year follow-up, found new sensitizations in 11/22 children receiving SIT vs. 22/22 children treated with placebo.²⁷ More recently, Pajno reported the results of a 6-year follow-up of 123 asthmatic children allergic to house dust mites⁸⁵: 75.4% of SIT children showed no new sensitization after the study, compared to

33.3% in the placebo group. Inal and colleagues found quite similar results among 147 children monosensitized to house dust mites: 75.3% without any new sensitization after 5 years in the SIT group, vs. 46.7% in the placebo group ($p = 0.02$).⁸⁶ A retrospective study of 8396 patients confirmed this protective effect of SIT on the development of further sensitizations.⁸⁷ Lastly, an open randomized controlled study by Marogna and colleagues showed the same protective effect: the study concerned 216 children with allergic rhinitis (with or without intermittent asthma), receiving drugs alone or drugs plus SLIT during 3 years. New sensitizations appeared in 34.8% of controls and in 3.1% of SLIT patients (OR 16.85, 95% CI: 5.73–49.13). The protective effect extended to bronchial hyperreactivity, which significantly decreased in the SLIT group.⁸⁸

These papers show that SIT not only induces the immune response towards the allergen used but also commits non-specifically the immune system to a system tolerating allergens. This effect could be due first to homology of structure between allergens. In that case SIT-induced immunomodulation could be specifically targeting common epitopes. Another hypothesis which is not demonstrated yet is the implication of non-specific bystander T cells that could also explain for example the efficacy of *P. pratense* extracts on other grass pollen allergies.^{26,76}

Some data suggest that early SIT could prevent from the occurrence of asthma. In the frame of the PAT Study, Möller and colleagues treated 205 children for seasonal rhinitis to birch and/or grass pollen.⁴⁶ Before starting the treatment, 20% of children displayed mild seasonal asthma. After 3

years of treatment, the actively treated children had significantly fewer asthma symptoms than untreated controls, with an odd ratio of 2.52 (95% CI: 1.3–5.1). A 10-year follow-up was recently reported: symptoms of asthma were still less frequent in SIT-treated patients compared to controls (OR: 2.5, 95% CI: 1.1–5.9), whereas no difference was found in bronchial responsiveness likely because of an improvement in the untreated subjects.⁴⁷ Unfortunately the study was not placebo-controlled, and these results must therefore be considered cautiously. Another study showed that SIT decreases the risk of non-specific bronchial hyperreactivity in rhinitic patients.⁸⁹ Novembre and colleagues enrolled 113 rhinitic children in a 3-year study of coseasonal sublingual grass pollen SIT, and found a 3.8 times more frequent development of asthma in the control group after 3 years (95% CI: 1.5–10).⁹⁰ Further studies must be prospectively performed to confirm that SIT protects rhinitic patients from asthma. Notably, it is noteworthy that these studies concerned pollen-induced seasonal asthma, which is not the most common form of asthma, and studies of the effect on SIT on the incidence of persistent non-seasonal asthma are needed. If true, however, this preventing effect of SIT on asthma would be a strong additive reason to treat patients with rhinitis. It would be interesting to separate analysis of results obtained in adults from these obtained in children since long-term immunomodulation might be age-dependent.

Safety aspects

The major risk of subcutaneous immunotherapy is severe anaphylaxis, with the paradox of giving a high-risk treatment in non-life threatening diseases. This risk must be considered for subcutaneous SIT at any time, even after several years of treatment. SIT induces local reactions which are expected and in the absence of which allergy and relevance of the treatment must be questioned. In case of loco-regional reactions, the doses must be reduced and the re-ascension of doses is more progressive. Syndromic reactions are frequent (rhinitis, conjunctivitis, asthma) and must also induce a dose reduction and more progressive re-ascension.⁹¹ Asthma attacks can be severe and mainly occur in asthmatics. It was clearly shown that the more severe the asthma the more frequent and severe the SIT-induced asthma attacks.⁹² Indeed in the Abramson's meta-analysis, among 426 treatments, 28 asthma or rhinitis episodes were reported, whereas only 7 anaphylaxis and urticaria occurred.⁴⁹ Kordash and Miller in the study of 13 fatal cases between 1992 and 1996 concluded that unstable asthma was a major risk factor.⁹³ Furthermore, asthma attack can be delayed to 30 min after the injection whereas other anaphylactic reactions generally occur in the minutes after treatment administration.⁹⁴ A poorly controlled asthma with frequent symptoms and important individual variations of FEV1 and peak-flow are considered as risk factors for SCIT-triggered asthma symptoms. This is why SIT should not be administered in asthmatic patients with a FEV1 below 70% of predicted values, and why patients must be kept under surveillance for at least 30 min in a place where a severe asthma attack can be treated. General reactions, from generalized urticaria to anaphylactic shock are rare.

In a report from the Mayo Clinic,⁹⁴ an incidence of 0.137% of general reactions was reported among a total of 79,593 injections. No death was reported in this series. Between 1985 and 1993, the FDA collected 0.6692 death per million of injection.⁹⁵ From a questionnaire sent to AAACI members, Reid and colleagues reported the occurrence of 17 deaths in 5 years.⁹⁶ Between 1959 and 1984, 46 deaths due to SIT were reported in USA.⁹⁷ Most deaths were due to dosage mistakes, spacing of injections, inadequate equipment, or unstable asthma. When it is performed with established security principles, SIT is well tolerated. Indeed Tabar and colleagues in house dust mite asthmatics reported local reactions in 10.5% of 419 cases and systemic reactions in 4.8% of patients (0.37% of injections) with a rapid response to treatment.⁹⁸ Luigi and colleagues⁹⁹ in another survey reported among 300,086 injections performed from 1968 to 1993 in 6319 asthmatic or rhinitic patients 184 (0.061%) systemic reactions in 131 (2.1%) patients. They comprised urticaria (59.3%), mild asthma (23.9%), asthma associated to urticaria (9.7%) and rhinitis (7.1%). A more recent retrospective study of accelerated SIT for various indications including venom allergy reported systemic reactions in 4.4% of injections.¹⁰⁰ Most of these reactions were mild, and were more frequent in asthmatics and during protocols using aqueous extracts. Moreno and colleagues¹⁰¹ reported 53 systemic reactions in 3.7% of 433 patients, showing a great stability over time of the incidence of these adverse reactions.

Sublingual immunotherapy is much safer. Post-marketing studies reported rare and mild adverse events in adults and children.^{102,103} In the update review by Passalacqua,³⁷ only 17 serious adverse reactions during SLIT were reported among all the controlled studies published between 2000 and 2006. No fatal event has been reported in any study. The rate of all adverse reactions was 17–60% in the SLIT-treated groups vs. 8–14% in the placebo-treated groups. They rarely induced an interruption of the treatment. The majority of these reactions was local, very mild (oral itching or swelling), and self-resolving. Interestingly, adverse effects were similar in children aged of 5 years or less. With sublingual grass allergen tablets, no severe side effect was reported in any study, and most adverse effects were observed at high non-recommended dosages (>500 IR).¹⁰⁴ However, it must be stressed that the risk of severe anaphylaxis, although exceptional, still exists with sublingual immunotherapy.¹⁰⁵

Guidelines

From the large number of studies published, in which the more important are detailed above, EAACI, AAAAI and WHO proposed guidelines concerning the use of immunotherapy, which were published in 1998,¹⁰⁶ revised in 2001,¹⁰⁷ 2007,¹⁰⁸ and 2008¹⁰⁹ in frame of the "Allergic Rhinitis and its Impact on Asthma" (ARIA) initiative. These guidelines are clearly stating the evidence-based use of SIT in the treatment of rhinitis and asthma. Sublingual immunotherapy is recommended for the treatment of pollen allergy in adults. Considering the lower proof of efficacy for house dust mites sublingual immunotherapy, SLIT is not recommended but "may be used" for patients with mite allergy.

For the European Academy of Allergy and Clinical Immunology,¹¹⁰ subcutaneous SIT is indicated in children above 5 years of age and adults during pollen-induced allergic diseases (grass, birch, ragweed, olive, parietaria, cypress), house dust mites and cat allergies when avoidance is not effective. As pollen avoidance is elusive and as the proof of efficacy of mite avoidance is limited, SIT can probably largely be considered. Multiple allergen therapy is not recommended. SLIT is indicated in patients above 5 years of age with allergic rhinoconjunctivitis and asthma, sensitive to birch, grasses, cypress, olive, parietaria or house dust mites. SLIT may be considered in patients insufficiently controlled by antiallergic drugs, such as antihistamines and/or inhaled (nasal or bronchial) steroids. The insufficient control of rhinitis and/or asthma relates to the persistence of symptoms and use of reliever medications despite the use of these controllers. However, as asthma has to be controlled to avoid adverse events of SIT as far as the injective route is considered, SIT is designed in that case to decrease the weight of controller treatments rather than as an add-on therapy. The 2007 ARIA update underlines the proved efficiency of specific immunotherapy, either sublingual or subcutaneous, to reduce bronchial hyperreactivity and its possibly protecting effect on asthma. The GINA guidelines¹¹¹ consider that SIT in asthma has a limited role. It must be limited to a single well-defined allergen, after strict environmental avoidance, with benefits weighed against risks. In the 90s British guidelines considered that SIT should not be administered in asthma.¹¹² In the recent actualization of ARIA guidelines¹⁰⁹ it is stated that SCIT can be considered in patients with asthma when a clinically significant allergen avoidance cannot be achieved. Experts warn about the potential risk of severe allergic reactions during SCIT and advise to fully discuss this risk with the patients. They do not recommend SLIT in asthma in routine practice, considering the proofs insufficient. Concerning primary prophylaxis, they require more studies to establish the interest of immunotherapy.

Conclusion

A large improvement of immunotherapy techniques and knowledge of indications have been made from the first use of this treatment in the early 1900s, so that SIT can confidently be considered as an evidence-based option for patients with allergic rhinitis and asthma. However, controlled studies have clearly defined the field of application of SIT, and have considerably restrained its application. Therefore, this treatment requires some very rigorous selection of extracts, of patients, and must be used by trained teams strictly aware of the risks taken. Recent non-invasive routes of administration are attractive notably for children, but a large effort of validation is still to be done.

Conflict of interest statement

Anaïs Pipet, Karine Botturi and Domitille Pinot have no conflict of interest. Antoine Magnan has received grant funding from GSK, MSD, Meda, Schering-Plough, Novartis, and Stallergènes. Daniel Vervloet and Antoine Magnan have

attended Conferences and Talks conducted by AstraZeneca, GlaxoSmithKline, Novartis, and MSD. Daniel Vervloet has served as consultant to GlaxoSmithKline and AstraZeneca. Antoine Magnan has served as consultant to AstraZeneca, Novartis, Meda AB, Allergbio, and Chiesi.

References

- Noon L. Prophylactic inoculation against hay fever. *Lancet* 1911;1:1572–3.
- Cooke R, Barnard J, Helbald S, Stull A. Serological evidence of immunity with coexisting sensitization in a type of human allergy (hay fever). *J Exp Med* 1935;62:733–50.
- Lichtenstein LM, Norman PS, Winkenwerder WL, Osler AG. In vitro studies of human ragweed allergy: changes in cellular and humoral activity associated with specific desensitization. *J Clin Invest* 1966;45(7):1126–36.
- Wachholz PA, Soni NK, Till SJ, Durham SR. Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy. *J Allergy Clin Immunol* 2003;112(5):915–22.
- Nouri-Aria KT, Wachholz PA, Francis JN, Jacobson MR, Walker SM, Wilcock LK, et al. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J Immunol* 2004;172(5):3252–9.
- van Neerven RJ, Wikborg T, Lund G, Jacobsen B, Brinch-Nielsen A, Arnved J, Ipsen H. Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4 + T cells by inhibiting serum-IgE-facilitated allergen presentation. *J Immunol* 1999 Sep 1;163(5):2944–52.
- Francis JN, James LK, Paraskevopoulos G, Wong C, Calderon MA, Durham SR, et al. Grass pollen immunotherapy: IL-10 induction and suppression of late responses precedes IgG4 inhibitory antibody activity. *J Allergy Clin Immunol* 2008;121(5):1120–5 [e2].
- Daeron M, Malbec O, Latour S, Arock M, Fridman WH. Regulation of high-affinity IgE receptor-mediated mast cell activation by murine low-affinity IgG receptors. *J Clin Invest* 1995;95(2):577–85.
- Passalacqua G, Albano M, Fregonese L, Riccio A, Pronzato C, Mela GS, et al. Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite-induced rhinoconjunctivitis. *Lancet* 1998;351(9103):629–32.
- Wilson DR, Nouri-Aria KT, Walker SM, Pajno GB, O'Brien F, Jacobson MR, et al. Grass pollen immunotherapy: symptomatic improvement correlates with reductions in eosinophils and IL-5 mRNA expression in the nasal mucosa during the pollen season. *J Allergy Clin Immunol* 2001;107(6):971–6.
- Nouri-Aria KT, Pilette C, Jacobson MR, Watanabe H, Durham SR. IL-9 and c-Kit+ mast cells in allergic rhinitis during seasonal allergen exposure: effect of immunotherapy. *J Allergy Clin Immunol* 2005;116(1):73–9.
- Secrist H, Chelen CJ, Wen Y, Marshall JD, Umetsu DT. Allergen immunotherapy decreases interleukin 4 production in CD4 + T cells from allergic individuals. *J Exp Med* 1993;178(6):2123–30.
- Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, et al. Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4 + T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon-gamma. *J Allergy Clin Immunol* 1996;97(6):1356–65.
- Jutel M, Pichler WJ, Skrbic D, Urwyler A, Dahinden C, Muller UR. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-gamma secretion in specific allergen-stimulated T cell cultures. *J Immunol* 1995;154(8):4187–94.

15. Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. *J Clin Invest* 1998; **102**(1):98–106.
16. Tran DQ, Ramsey H, Shevach EM. Induction of FOXP3 expression in naive human CD4+FOXP3 T cells by T-cell receptor stimulation is transforming growth factor-beta dependent but does not confer a regulatory phenotype. *Blood* 2007; **110**(8): 2983–90.
17. Ling EM, Smith T, Nguyen XD, Pridgeon C, Dallman M, Arbery J, et al. Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. *Lancet* 2004; **363**(9409):608–15.
18. Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Cramer R, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004; **199**(11):1567–75.
19. Fancisi JN, Till SJ, Durham SR. Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol* 2003; **111**(6):1255–61.
20. Jutel M, Akdis M, Budak F, Aebischer-Casaulta C, Wrzyszczyk M, Blaser K, et al. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003; **33**(5): 1205–14.
21. Mamelessier E, Birnbaum J, Dupuy P, Vervloet D, Magnan A. Ultra-rush VIT induces differential T cell activation and regulatory patterns according to the severity of allergy. *Clin Exp Allergy* 2006; **36**:704–13.
22. Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces Foxp3-expressing CD4(+)CD25(+) cells in the nasal mucosa. *J Allergy Clin Immunol* 2008; **121**:1467–72.
23. Pilette C, Nouri-Aria KT, Jacobson MR, Wilcock LK, Detry B, Walker SM, et al. Grass pollen immunotherapy induces an allergen-specific IgA2 antibody response associated with mucosal TGF-beta expression. *J Immunol* 2007; **178**(7): 4658–66.
24. Bohle B, Kinaciyan T, Gerstmayr M, Radakovichs A, Jahn-Schmid B, Ebner C. Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. *J Allergy Clin Immunol* 2007 Sep; **120**(3):707–13.
25. Rocklin RE, Sheffer AL, Greineder DK, Melmon KL. Generation of antigen-specific suppressor cells during allergy desensitization. *N Engl J Med* 1980; **302**(22):1213–9.
26. Botturi K, Vervloet D, Magnan A. T cells and allergens relationships: are they that specific? *Clin Exp Allergy* 2007; **37**(8): 1121–3.
27. Des Roches A, Paradis L, Menardo JL, Bouges S, Daures JP, Bousquet J. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. VI. Specific immunotherapy prevents the onset of new sensitizations in children. *J Allergy Clin Immunol* 1997; **99**(4):450–3.
28. Hammad H, Lambrecht BN. Dendritic cells and epithelial cells: linking innate and adaptive immunity in asthma. *Nat Rev Immunol* 2008; **8**(3):193–204.
29. Akoum H, Tsicopoulos A, Vorng H, Wallaert B, Dessaint JP, Joseph M, et al. Venom immunotherapy modulates interleukin-4 and interferon-gamma messenger RNA expression of peripheral T lymphocytes. *Immunology* 1996; **87**(4):593–8.
30. Magnan A, Marin V, Mely L, Birnbaum J, Romanet S, Bongrand P, et al. Venom immunotherapy induces monocyte activation. *Clin Exp Allergy* 2001; **31**(8):1303–9.
31. Hamid Q, Naseer T, Minshall EM, Song YL, Boguniewicz M, Leung DY. In vivo expression of IL-12 and IL-13 in atopic dermatitis. *J Allergy Clin Immunol* 1996; **98**(1):225–31.
32. Creticos PS, Schroeder JT, Hamilton RG, Balcer-Whaley SL, Khattignavong AP, Lindblad R, et al. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. *N Engl J Med* 2006; **355**(14):1445–55.
33. Lombardi V, Van Overtvelt L, Horiot S, Moussu H, Chabre H, Louise A, Balazuc AM, Mascarell L, Moingeon P. Toll-like receptor 2 agonist Pam3CSK4 enhances the induction of antigen-specific tolerance via the sublingual route. *Clin Exp Allergy*; 2008 Jul 17 [Epub ahead of print].
34. Norman PS. Is there a role for immunotherapy in the treatment of asthma? Yes. *Am J Respir Crit Care Med* 1996; **154**(5): 1225–6.
35. Barnes PJ. Is there a role for immunotherapy in the treatment of asthma? No. *Am J Respir Crit Care Med* 1996; **154**(5): 1227–8.
36. Calderon MA, Alves B, Jacobson M, Hurwitz B, Sheikh A, Durham S. Allergen injection immunotherapy for seasonal allergic rhinitis. *Cochrane Database Syst Rev* 2007; **(1)**: CD001936.
37. Passalacqua G, Durham SR. Allergic rhinitis and its impact on asthma update: allergen immunotherapy. *J Allergy Clin Immunol* 2007; **119**(4):881–91.
38. Frew AJ, Powell RJ, Corrigan CJ, Durham SR. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2006; **117**(2):319–25.
39. Walker SM, Pajno GB, Lima MT, Wilson DR, Durham SR. Grass pollen immunotherapy for seasonal rhinitis and asthma: a randomized, controlled trial. *J Allergy Clin Immunol* 2001; **107**(1):87–93.
40. Powell RJ, Frew AJ, Corrigan CJ, Durham SR. Effect of grass pollen immunotherapy with Alutard SQ on quality of life in seasonal allergic rhinoconjunctivitis. *Allergy* 2007; **62**(11): 1335–8.
41. Mosbech H, Osterballe O. Does the effect of immunotherapy last after termination of treatment? Follow-up study in patients with grass pollen rhinitis. *Allergy* 1988; **43**(7): 523–9.
42. Eng PA, Borer-Reinhold M, Heijnen IA, Gnehm HP. Twelve-year follow-up after discontinuation of preseasonal grass pollen immunotherapy in childhood. *Allergy* 2006; **61**(2):198–201.
43. Des Roches A, Paradis L, Knani J, Hejjoui A, Dhivert H, Chanez P, et al. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. V. Duration of the efficacy of immunotherapy after its cessation. *Allergy* 1996; **51**(6): 430–3.
44. Naclerio RM, Proud D, Moylan B, Balcer S, Freidhoff L, Kagey-Sobotka A, et al. A double-blind study of the discontinuation of ragweed immunotherapy. *J Allergy Clin Immunol* 1997; **100**(3):293–300.
45. Durham SR, Walker SM, Varga EM, Jacobson MR, O'Brien F, Noble W, et al. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 1999; **341**(7):468–75.
46. Moller C, Dreborg S, Ferdousi HA, Halken S, Host A, Jacobsen L, et al. Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). *J Allergy Clin Immunol* 2002; **109**(2): 251–6.
47. Jacobsen L, Niggemann B, Dreborg S, Ferdousi HA, Halken S, Host A, et al. Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study. *Allergy* 2007; **62**(8):943–8.
48. Abramson MJ, Puy RM, Weiner JM. Is allergen immunotherapy effective in asthma? A meta-analysis of randomized controlled trials. *Am J Respir Crit Care Med* 1995; **151**(4): 969–74.
49. Abramson M, Puy R, Weiner J. Immunotherapy in asthma: an updated systematic review. *Allergy* 1999; **54**(10):1022–41.

50. Abramson MJ, Puy RM, Weiner JM. Allergen immunotherapy for asthma. *Cochrane Database Syst Rev* 2000;(2):CD001186.
51. Abramson MJ, Puy RM, Weiner JM. Allergen immunotherapy for asthma. *Cochrane Database Syst Rev* 2003;(4):CD001186.
52. Basomba A, Tabar AI, de Rojas DH, Garcia BE, Alamar R, Olaguibel JM, et al. Allergen vaccination with a liposome-encapsulated extract of *Dermatophagoides pteronyssinus*: a randomized, double-blind, placebo-controlled trial in asthmatic patients. *J Allergy Clin Immunol* 2002;109(6):943–8.
53. Pifferi M, Baldini G, Marrazzini G, Baldini M, Ragazzo V, Pietrobelli A, et al. Benefits of immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract in asthmatic children: a three-year prospective study. *Allergy* 2002;57(9):785–90.
54. Garcia-Robaina JC, Sanchez I, de la Torre F, Fernandez-Caldas E, Casanovas M. Successful management of mite-allergic asthma with modified extracts of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* in a double-blind, placebo-controlled study. *J Allergy Clin Immunol* 2006;118(5):1026–32.
55. Arvidsson MB, Lowhagen O, Rak S. Effect of 2-year placebo-controlled immunotherapy on airway symptoms and medication in patients with birch pollen allergy. *J Allergy Clin Immunol* 2002;109(5):777–83.
56. Roberts G, Hurley C, Turcanu V, Lack G. Grass pollen immunotherapy as an effective therapy for childhood seasonal allergic asthma. *J Allergy Clin Immunol* 2006;117(2):263–8.
57. Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* 2005;60(1):4–12.
58. Penagos M, Compalati E, Tarantini F, Baena-Cagnani R, Huerta J, Passalacqua G, et al. Efficacy of sublingual immunotherapy in the treatment of allergic rhinitis in pediatric patients 3 to 18 years of age: a meta-analysis of randomized, placebo-controlled, double-blind trials. *Ann Allergy Asthma Immunol* 2006;97(2):141–8.
59. Penagos M, Passalacqua G, Compalati E, Baena-Cagnani CE, Orozco S, Pedroza A, Canonica GW. Metaanalysis of the efficacy of sublingual immunotherapy in the treatment of allergic asthma in pediatric patients, 3 to 18 years of age. *Chest* 2008 March;133(3):599–609.
60. Calamita Z, Saconato H, Pela AB, Atallah AN. Efficacy of sublingual immunotherapy in asthma: systematic review of randomized-clinical trials using the Cochrane Collaboration method. *Allergy* 2006;61(10):1162–72.
61. Pradalier A, Basset D, Claudel A, Couturier P, Wessel F, Galvain S, et al. Sublingual-swallow immunotherapy (SLIT) with a standardized five-grass-pollen extract (drops and sublingual tablets) versus placebo in seasonal rhinitis. *Allergy* 1999;54(8):819–28.
62. La Rosa M, Ranno C, Andre C, Carat F, Tosca MA, Canonica GW. Double-blind placebo-controlled evaluation of sublingual-swallow immunotherapy with standardized *Parietaria judaica* extract in children with allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 1999;104(2 Pt 1):425–32.
63. Voltolini S, Modena P, Minale P, Bignardi D, Troise C, Puccinelli P, et al. Sublingual immunotherapy in tree pollen allergy. Double-blind, placebo-controlled study with a biologically standardised extract of three pollens (alder, birch and hazel) administered by a rush schedule. *Allergol Immunopathol (Madr)* 2001;29(4):103–10.
64. Valovirta E, Jacobsen L, Ljorring C, Koivikko A, Savolainen J. Clinical efficacy and safety of sublingual immunotherapy with tree pollen extract in children. *Allergy* 2006;61(10):1177–83.
65. Alvarez-Cuesta E, Berges-Gimeno P, Gonzalez-Mancebo E, Fernandez-Caldas E, Cuesta-Herranz J, Casanovas M. Sublingual immunotherapy with a standardized cat dander extract: evaluation of efficacy in a double blind placebo controlled study. *Allergy* 2007;62(7):810–7.
66. Nelson HS, Oppenheimer J, Vatsia GA, Buchmeier A. A double-blind, placebo-controlled evaluation of sublingual immunotherapy with standardized cat extract. *J Allergy Clin Immunol* 1993;92:229–36.
67. Aberer W, Hawranek T, Reider N, Schuster C, Sturm G, Kränke B, Immunoglobulin E. G antibody profile to grass pollen allergens during a short course of sublingual immunotherapy. *J Invest Allergol Clin Immunol* 2007;17(3):131–6.
68. Laffer S, Spitzauer S, Susani M, Pairleitner H, Schweiger C, Grönlund H, Menz G, Pauli G, Ishii T, Nolte H, Ebner C, Sehon AH, Kraft D, Eichler HG, Valenta R. Comparison of recombinant timothy grass pollen allergens with natural extract for diagnosis of grass pollen allergy in different populations. *J Allergy Clin Immunol* 1996;98(3):652–8.
69. Kleine-Tebbe J, Ribel M, Herold DA. Safety of a SQ-standardised grass allergen tablet for sublingual immunotherapy: a randomized, placebo-controlled trial. *Allergy* 2006;61(2):181–4.
70. Malling HJ, Lund L, Ipsen H, Poulsen L. Safety and immunological changes during sublingual immunotherapy with standardized quality grass allergen tablets. *J Invest Allergol Clin Immunol* 2006;16(3):162–8.
71. Calderon M, Essendrop M. Specific immunotherapy with high dose SQ standardized grass allergen tablets was safe and well tolerated. *J Invest Allergol Clin Immunol* 2006;16(6):338–44.
72. Ibanez MD, Kaiser F, Knecht R, Armentia A, Schopfer H, Tholstrup B, et al. Safety of specific sublingual immunotherapy with SQ standardized grass allergen tablets in children. *Pediatr Allergy Immunol* 2007;18(6):516–22.
73. Dahl R, Kapp A, Colombo G, de Monchy JG, Rak S, Emminger W, et al. Efficacy and safety of sublingual immunotherapy with grass allergen tablets for seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2006;118(2):434–40.
74. Rak S, Yang WH, Pedersen MR, Durham SR. Once-daily sublingual allergen-specific immunotherapy improves quality of life in patients with grass pollen-induced allergic rhinoconjunctivitis: a double-blind, randomised study. *Qual Life Res* 2007;16(2):191–201.
75. Calderon MA, Birk AO, Andersen JS, Durham SR. Prolonged preseasonal treatment phase with Grazax sublingual immunotherapy increases clinical efficacy. *Allergy* 2007;62(8):958–61.
76. Didier A, Malling HJ, Worm M, Horak F, Jager S, Montagut A, et al. Optimal dose, efficacy, and safety of once-daily sublingual immunotherapy with a 5-grass pollen tablet for seasonal allergic rhinitis. *J Allergy Clin Immunol* 2007;120(6):1338–45.
77. Passalacqua G, Pasquali M, Ariano R, Lombardi C, Giardini A, Baiardini I, et al. Randomized double-blind controlled study with sublingual carbamylated allergoid immunotherapy in mild rhinitis due to mites. *Allergy* 2006;61(7):849–54.
78. Pham-Thi N, Scheinmann P, Fadel R, Combebias A, Andre C. Assessment of sublingual immunotherapy efficacy in children with house dust mite-induced allergic asthma optimally controlled by pharmacologic treatment and mite-avoidance measures. *Pediatr Allergy Immunol* 2007;18(1):47–57.
79. Quirino T, Iemoli E, Siciliani E, Parmiani S, Milazzo F. Sublingual versus injective immunotherapy in grass pollen allergic patients: a double blind (double dummy) study. *Clin Exp Allergy* 1996;26:1253–61.
80. Khinchi MS, Poulsen LK, Carat F, Andre C, Hansen AB, Malling HJ. Clinical efficacy of sublingual and subcutaneous birch pollen allergen-specific immunotherapy: a randomized,

- placebo-controlled, double-blind, double-dummy study. *Allergy* 2004;**59**(1):45–53.
81. Mauro M, Russello M, Incorvaia C, Gazzola GB, Di Cara G, Frati F. Comparison of efficacy, safety and immunologic effects of subcutaneous and sublingual immunotherapy in birch pollinosis: a randomized study. *Eur Ann Allergy Clin Immunol* 2007;**39**(4):119–22.
 82. Mungan D, Misirligil Z, Gurbuz L. Comparison of the efficacy of subcutaneous and sublingual immunotherapy in mite-sensitive patients with rhinitis and asthma – a placebo controlled study. *Ann Allergy Asthma Immunol* 1999;**82**:485–90.
 83. Lombardi C, Gani F, Landi M, Falagiani P, Bruno M, Canonica GW, et al. Quantitative assessment of the adherence to sublingual immunotherapy. *J Allergy Clin Immunol* 2004;**113**(6):1219–20.
 84. Pajno GB, Vita D, Caminiti L, Arrigo T, Lombardo F, Incorvaia C, et al. Children's compliance with allergen immunotherapy according to administration routes. *J Allergy Clin Immunol* 2005;**116**(6):1380–1.
 85. Pajno GB, Barberio G, De Luca F, Morabito L, Parmiani S. Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. *Clin Exp Allergy* 2001;**31**(9):1392–7.
 86. Inal A, Altintas DU, Yilmaz M, Karakoc GB, Kendirli SG, Sertdemir Y. Prevention of new sensitizations by specific immunotherapy in children with rhinitis and/or asthma monosensitized to house dust mite. *J Investig Allergol Clin Immunol* 2007;**17**(2):85–91.
 87. Purello-D'Ambrosio F, Gangemi S, Merendino RA, Isola S, Puccinelli P, Parmiani S, et al. Prevention of new sensitizations in monosensitized subjects submitted to specific immunotherapy or not. A retrospective study. *Clin Exp Allergy* 2001;**31**(8):1295–302.
 88. Marogna M, Tomassetti D, Bernasconi A, Colombo F, Massolo A, Businco AD, Canonica GW, Passalacqua G, Tripodi S. Preventive effects of sublingual immunotherapy in childhood: an open randomized controlled study. *Ann Allergy Asthma Immunol* 2008;**101**(2):206–11.
 89. Grembiale RD, Camporota L, Naty S, Tranfa CM, Djukanovic R, Marsico SA. Effects of specific immunotherapy in allergic rhinitic individuals with bronchial hyperresponsiveness. *Am J Respir Crit Care Med* 2000;**162**(6):2048–52.
 90. Novembre E, Galli E, Landi F, Caffarelli C, Pifferi M, De Marco E, et al. Coseasonal sublingual immunotherapy reduces the development of asthma in children with allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2004;**114**(4):851–7.
 91. Malling H, Immunotherapy BW. Position paper of the European Academy of Allergology and Clinical Immunology. *Allergy* 1993;**48**(Suppl. 14):9–35.
 92. Bousquet J, Hejjaoui A, Dhivert H, Clauzel AM, Michel FB. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. Systemic reactions during the rush protocol in patients suffering from asthma. *J Allergy Clin Immunol* 1989;**83**(4):797–802.
 93. Kordash T, Miller J. Allergenic extracts used in immunotherapy fatalities. *J Allergy Clin Immunol* 1997;**99**(S67).
 94. Valyasevi M, Yocum M, Gosselin V, Hunt L. Systemic reactions to immunotherapy at the Mayo Clinic. *J Allergy Clin Immunol* 1997;**99**(S66).
 95. Turkeltaub P. Deaths associated with allergenic extracts. *FDA Med Bull* 1994;**24**(May).
 96. Reid MJ, Lockey RF, Turkeltaub PC, Platts-Mills TA. Survey of fatalities from skin testing and immunotherapy 1985–1989. *J Allergy Clin Immunol* 1993;**92**(1 Pt 1):6–15.
 97. Lockey RF, Benedict LM, Turkeltaub PC, Bukantz SC. Fatalities from immunotherapy (IT) and skin testing (ST). *J Allergy Clin Immunol* 1987;**79**(4):660–77.
 98. Tabar AI, Garcia BE, Rodriguez A, Olaguibel JM, Muro MD, Quirce S. A prospective safety-monitoring study of immunotherapy with biologically standardized extracts. *Allergy* 1993;**48**(6):450–3.
 99. Luigi A, Senna G, Mezzelani P, Pappalardo G. Safety of specific immunotherapy: a retrospective study. *J Investig Allergol Clin Immunol* 1994;**4**(5):250–4.
 100. Møllerup MT, Hahn GW, Poulsen LK, Malling H. Safety of allergen-specific immunotherapy. Relation between dosage regimen, allergen extract, disease and systemic side-effects during induction treatment. *Clin Exp Allergy* 2000;**30**(10):1423–9.
 101. Moreno C, Cuesta-Herranz J, Fernandez-Tavora L, Alvarez-Cuesta E. Immunotherapy safety: a prospective multi-centric monitoring study of biologically standardized therapeutic vaccines for allergic diseases. *Clin Exp Allergy* 2004;**34**(4):527–31.
 102. Lombardi C, Gargioni S, Melchiorre A, Tiri A, Falagiani P, Canonica GW, et al. Safety of sublingual immunotherapy with monomeric allergoid in adults: multicenter post-marketing surveillance study. *Allergy* 2001;**56**(10):989–92.
 103. Rienzo VD, Minelli M, Musarra A, Sambugaro R, Pecora S, Canonica WG, et al. Post-marketing survey on the safety of sublingual immunotherapy in children below the age of 5 years. *Clin Exp Allergy* 2005;**35**(5):560–4.
 104. Larsen TH, Poulsen LK, Melac M, Combebias A, Andre C, Malling HJ. Safety and tolerability of grass pollen tablets in sublingual immunotherapy – a phase-1 study. *Allergy* 2006;**61**(10):1173–6.
 105. Dunskey EH, Goldstein MF, Dvorin DJ, Belecanech GA. Anaphylaxis to sublingual immunotherapy. *Allergy* 2006;**61**(10):1235.
 106. Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 1998;**102**(4 Pt 1):558–62.
 107. Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001;**108**(Suppl. 5):S147–334.
 108. Cruz AA, Popov T, Pawankar R, Annesi-Maesano I, Fokkens W, Kemp J, et al. Common characteristics of upper and lower airways in rhinitis and asthma: ARIA update, in collaboration with GA(2)LEN. *Allergy* 2007;**62**(Suppl. 84):1–41.
 109. ARIA: 2008 update. *Allergy* 2008;**63**(Suppl. 86):8–16.
 110. Alvarez-Cuesta E, Bousquet J, Canonica GW, Durham SR, Malling HJ, Valovirta E. Standards for practical allergen-specific immunotherapy. *Allergy* 2006;**61**(Suppl. 82):1–20.
 111. www.ginasthma.org.
 112. British Thoracic Society Scottish Intercollegiate Guidelines Network. British guideline on the management of asthma. *Thorax* 2008;**63**(Suppl. 4):iv1–121.