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REVIEW

# Mechanisms of immunotherapy to aeroallergens

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**Summary**

Allergen immunotherapy is allergen-specific, allergen dose- and time-dependent and is associated with long-term clinical and immunological tolerance that persists for years after discontinuation. Successful immunotherapy is accompanied by the suppression of numbers of T-helper 2 (Th2) effector cells, eosinophils, basophils, c-kit+mast cells and neutrophils infiltration in target organs, induction of IL-10 and/or TGF-β+Treg cells and increases in 'protective' non-inflammatory blocking antibodies, particularly IgG4 and IgA2 subclasses with inhibitory activity. These events are accompanied by a reduction and/or a redirection of underlying antigen-specific Th2-type T cell-driven hypersensitivity to the allergen(s) used for therapy. This suppression occurs within weeks or months as a consequence of the appearance of a population of regulatory T cells that exert their effects by mechanisms involving cell–cell contact, but also by the release of cytokines such as IL-10 (increases IgG4) and TGF-β (increases specific IgA). The more delayed-in-time appearance of antigen-specific T-helper 1 responses and alternative mechanisms such as Th2 cell anergy and/or apoptosis may also be involved. The mechanisms of sublingual immunotherapy are similar to those following a subcutaneous administration of allergen, whereas it is likely that additional events following antigen presentation in the sublingual mucosa and regional lymph nodes are involved. These insights have resulted in novel approaches and portend future biomarkers that may be surrogate or predictive of the clinical response to treatment.

## Introduction

Conventional allergen-specific immunotherapy (SIT) involves the repeated administration of sensitizing allergen subcutaneously. Incremental allergen doses are given for a period of 8–16 weeks, followed by monthly maintenance injections for a period of 3–5 years [1]. Recently, sublingual allergen-specific immunotherapy (SLIT) has emerged as an effective and safer alternative route of immunotherapy for IgE-mediated hypersensitivity against aeroallergens. To date, SIT is the only immunomodulatory intervention that induces allergen-specific immunological tolerance [1, 2]. Injection allergen-SIT for seasonal pollinosis is associated with the amelioration of seasonal symptoms, bronchial hyperresponsiveness (BHR) and a reduced requirement for rescue medication [3–5]. Clinical responsiveness to SIT has been shown to exceed the duration of SIT treatment by several years, a clear advantage over the use of anti-IgE or anti-allergic drugs [6, 7]. SIT reduces the onset of new sensitizations in children [8] and there is preliminary evidence that SIT may prevent the progression of allergic rhinitis to physician-diagnosed asthma [9, 10]. In this article, we present a review of the immunological mechanisms of subcutaneous (SCIT) and SLIT. Furthermore, the mechanisms of long-term clinical benefits and potential biomarkers of clinical efficacy are evaluated.

## Effects of injection immunotherapy on early and late allergic responses

The suppression of the late-phase allergic responses in the skin [11], nose [12] and the lung [13] has been reported following allergen-SIT. Several studies have reported inhibition of the early and the late response following an intradermal allergen challenge [6, 12, 14–16]. Reduced numbers of T lymphocytes and suppression of eosinophil, basophil and neutrophil infiltration in skin biopsies have been reported following allergen-SIT [14, 15]. Furthermore, suppression of the influx of effector cells in the skin

after allergen challenge may explain the observed decrease in the size of the late cutaneous response. Despite a significant reduction of mast cell numbers in the skin, the early allergic response was only partially suppressed following SIT [6]. The time-course of the early and late allergic inflammatory response in the skin has been studied in detail following timothy grass pollen SIT [17]. Interestingly, suppression of the late allergic response to an intradermal allergen challenge was significant within 2 weeks and evident even at low allergen doses. In contrast, a modest but significant reduction in the early allergic response occurred later and only after achieving maintenance doses following 8–12 weeks of up-dosing therapy. Similarly, allergen-induced early and late nasal responses are suppressed following immunotherapy for grass pollen [18] and cat allergens [19], whereas late asthmatic responses are inhibited following mite [13] and birch pollen SIT [20].

### Cellular responses following allergen-specific immunotherapy

A schema for the underlying mechanisms of allergen-SIT is presented in Fig. 1. SCIT is associated with changes in cellular and humoral responses with a significant reduction of tissue infiltrating inflammatory effector cells. The induction of long-term tolerance after immunotherapy is associated with a shift in the ratio of T-helper 2 (Th2) and T-helper 1 (Th1) cytokines. However, not all studies have confirmed these findings. Although increases in Th1 cytokines in peripheral blood cells have been reported following allergen-SIT [21, 22], other studies failed to report any changes in Th1 cytokine production by these cells [23, 24]. However, the change in the ratio of Th1 and Th2 cytokines has been observed in local target organs [23, 25] and may control allergen-induced allergic responses by inhibiting the pro-inflammatory effects of dendritic cells (DCs) [26, 27].

The influence of allergen-SIT on peripheral DCs has been demonstrated recently [27]. Allergen-SIT increased DC TLR9-mediated innate immune function, which was previously impaired at baseline in allergic subjects. In this study, a robust innate immune response from isolated pDCs was re-established among house dust mite (HDM)-allergic subjects undergoing allergen-SIT, resulting in a three- to fivefold increase in IFN-α production in response to CpG stimulation [27]. Furthermore, the proportions of peripheral blood pDCs, but not mDCs, are significantly reduced after the initiation of Hymenoptera venom-SIT. This was associated with changes in the expression of function-associated surface molecules

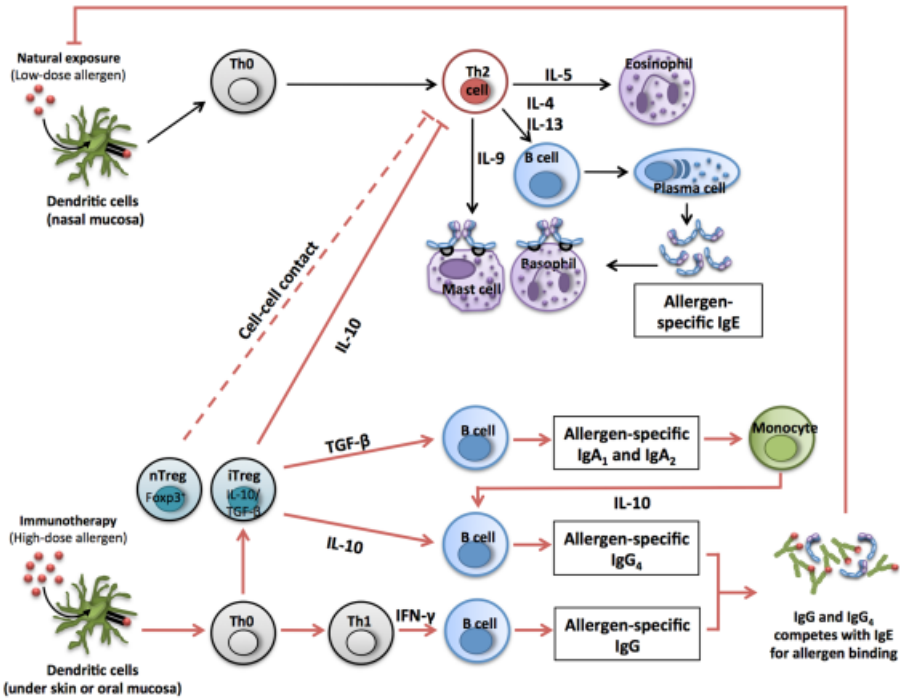


Fig. 1. Immunological mechanisms of immunotherapy to aeroallergens. Low-dose and repeated allergen exposure at mucosal surfaces in atopic individuals drives type I IgE-mediated allergic responses. High-allergen dose through a subcutaneous or a sublingual route results in the shift of T cell polarization from a T helper 2 (Th2) to a T helper 1 (Th1) response. This is accompanied by an increase in the ratio of Th1 cytokines (IFN-γ, IL-12) to Th2 cytokines (IL-4, IL-5 and IL-13). The induction of T regulatory cells [inducible Treg cells (iTreg) and natural Treg cells (nTreg)] and cytokines such as IL-10 and TGF-β following immunotherapy play an important role in suppressing Th1 and Th2 responses and contributes towards the induction of allergen-specific IgA1, IgA2 and in particular IgG4 antibodies with inhibitory activity. IgG4 antibodies are able to suppress FcεRI- and CD23-mediated IgE-facilitated allergen presentation and basophil histamine release.

such as CD32, CD40 and TLR2 on DCs during allergen-SIT. Altogether, numeric and phenotypic changes of blood DCs may contribute to a suppression of allergic inflammatory response during SIT [28].

Peripheral T cell tolerance following SIT is characterized by the induction of allergen-specific regulatory T cells (Treg cell; IL-10-producing T cells and CD4+ CD25+ T cells). The regulatory function of IL-10/TGF-β and CD4+CD25+ T cells has been reported to overlap. Induction of cytokines such as IL-10 and TGF-β by CD4+CD25+ following SIT has been reported in several studies [21, 24, 29, 30]. These cells have been shown to suppress antigen-driven proliferative T cell responses and Th2 cytokine release in an IL-10-dependent manner [21]. Several novel biomarkers that include increased expression of the intracellular transcription factor FoxP3 together with increased cell surface expression of the receptor CD25 and reduced CD127 have been shown to characterize regulatory T cells. Phenotypic regulatory T cells (CD4+CD25+CD127lo) remained unchanged following a comprehensive, longitudinal study of birch pollen SIT [31]. Furthermore, the proportion of CD25+ and CD25hi populations within peripheral CD4 T cells did not change in patients treated with birch pollen-SIT compared with untreated birch-allergic controls [32]. In contrast, increased functional Treg cell activity has been identified following an *ex vivo* allergen stimulation of T cell populations in peripheral blood. Thus, elevated levels of IL-10 production in peripheral blood mononuclear cell (PBMC) cultures have been demonstrated in patients treated with SIT compared with placebo-treated controls [17]. The time course of increases in IL-10 occurred early, within 2 weeks, and paralleled the suppression of the associated, T cell-driven, cutaneous late response. Furthermore, increases in the frequency of antigen-specific IL-10+ cells have been demonstrated by the EliSpot assay [31]. In the same study, enriched peripheral IL-10+CD4+ T cells using IL-10 secretion assay suppressed proliferative responses in an IL-10-dependent manner.

Moreover, TGF-β has been shown to have a potent regulatory property. It is proven essential for the maintenance of immunological self-tolerance in the CD4 T cell compartment. TGF-β inhibits the differentiation of Th1 and Th2 cells by inhibiting the expression of transcription factors T-BET and GATA-3 [33, 34]. The lack of Th1 and Th2 polarization during T cell differentiation results in the generation of Treg cells [35]. TGF-β induces the expression of FOXP3, which promotes the induction of T cells with a regulatory phenotype [36]. It also induces CTLA-4 expression in Treg cells [37, 38]. TGF-β modulates IgE, FcεRI expression on Langerhans cells and is a class switch factor for non-inflammatory IgA antibodies [39]. These findings suggest its role in inducing peripheral tolerance.

Furthermore, recent studies have demonstrated elevated numbers of IL-10+ and TGF-β+ T cells and FoxP3+CD4+ and FoxP3+CD25+ phenotypic regulatory T cells within the nasal mucosa [40, 41]. These local increases in phenotypic regulatory T cells within the target organ paralleled clinical improvement and reduced inflammatory allergic responses (a reduction in mast cells [39], basophils [42] and eosinophils [43] in the nasal mucosa).

More recently, HDM-SIT has been associated with the induction of CD8+CD25+FoxP3+ Treg cells [44]. PBMCs isolated from healthy control subjects and from HDM-sensitive asthmatic patients before treatment, 6 and 12 months showed a significant increase in CD8+Foxp3+ Treg cells expressing intracellular IL-10 and granzyme B. Co-stimulation of PBMCs with Pam3CSK4 (a synthetic TLR2 ligand) and *Dermatophagoides pteronyssinus* II expanded the CD8+CD25+Foxp3+ Treg population and inhibited *D. pteronyssinus* 2-induced IL-4 production [44].

### Immunoglobulin responses following allergen-specific immunotherapy

In atopic individuals, exposure to relevant allergen results in an increased concentrations in allergen-specific IgE in the serum and in the local target organs. van Ree and colleagues demonstrated a transient increase in rye grass pollen (Lol p 1, 2, 3 and 5)-specific IgE in patients during the season, followed by a gradual decrease over time [45]. SIT in patients with hayfever is associated with transient early increases in allergen-specific IgE, followed by blunting of seasonal increases in IgE [1, 46, 47]. In a proportion of individuals who received birch pollen-SIT, low concentrations of IgE antibodies to previously unrecognized proteins were identified [48]. The clinical relevance of these new sensitizations is doubtful as they paralleled clinical improvement with no apparent adverse events.

Quantitative measurements of allergen-specific IgG subclasses in SIT-treated patients have revealed increases in allergen-specific IgG1 and IgG4 antibody concentrations in the serum and in the local target organs [21, 49, 50]. Changes in IgG2 and IgG3 levels were not significant [51]. Despite these increases in the levels of allergen-specific antibodies, many studies have failed to show a correlation between allergen-specific IgG1 and IgG4 antibodies and clinical efficacy.

The possible functional relevance of these allergen-specific inhibitory IgG1 and IgG4 antibodies has been illustrated in several studies. Bet v1-specific IgG1 and IgG4 antibodies from SIT-treated patients inhibited basophil histamine release in an antigen-specific fashion [52–54]. These monoclonal Bet v1-specific IgG1 and IgG4 could compete with Bet v1-specific IgE and prevent its interaction with Bet v1 allergen. In a murine model of allergy, the inhibitory activities of IgG were shown to be mediated via the FcγRIIB receptor. Incubation of IgE, allergen and IgG immune complexes with mouse mast cells resulted in the juxtaposition or the co-aggregation of

the FcγRIIB and FcεRI resulting in the suppression of mast cell degranulation [55]. Additionally, inhibition of basophil histamine release was demonstrated using the recombinant chimeric Fcγ–Fcε construct that potentiates FcγRIIb and FcεRI co-aggregation on human basophils *in vitro* [56]. This inhibitory effect was dependent on immunoreceptor tyrosine-based inhibitory motif phosphorylation, resulting in the activation of intracellular phosphatases and counterbalance of the influence of immunoreceptor-based activation motifs present within the intracellular tail of the FcεRIγ expressed on human basophils [57]. In contrast, in a human model [58], blockade of downstream signalling of FcγRIIB with a monoclonal antibody directed against CD32 did not block IgG-mediated inhibitory activity following birch pollen-SIT, which implies direct competition with IgE for an allergen rather than a mechanism involving downstream inhibition of the IgE receptor signalling pathway as observed in the murine model.

van Neerven and colleagues demonstrated that the serum obtained from subjects following birch pollen-SIT was able to inhibit IgE-facilitated allergen presentation by B cells to an allergen-specific T cell clone, resulting in decreased T cell proliferation and reduced cytokine production by birch pollen-specific T cells [59]. Wachholz and colleagues showed that the serum obtained from subjects who participated in a randomized double-blind placebo-controlled trial of grass pollen-SIT could inhibit IgE-facilitated allergen presentation to a grass-specific T cell clone [60]. Moreover, using a simplified assay where allergen-IgE complexes bound to FcεRII on the surface of B cells were detected by flow cytometry (IgE-FAB), it was shown that the vigour of proliferative responses by T cell clones reflects the binding of these complexes [60]. This was confirmed in patients following allergen-SIT for birch allergy [61]. Nouri-Aria and colleagues further confirmed increases in allergen-specific IgG4 antibodies in patients treated with grass pollen-SIT. In the same study, post-allergen-SIT serum was able to inhibit IgE-facilitated allergen binding. This serum inhibitory activity was shown to co-purify with IgG4-containing fractions following affinity chromatography [62]. These findings suggest that IgG antibodies are involved in the underlying mechanisms of successful SIT. Hence, the measurement of inhibition of IgE-facilitated allergen binding has been utilized as a potential surrogate marker of successful allergen-SIT in several clinical trials [17, 61, 63–65].

Immunoglobulin isotype class switching, in particular for IgG4 and IgE, is dependent on Th2 cytokines (IL-4 and IL-13) and cognate interaction of CD40 on Th cells and the CD40 ligand on B cells [66–68]. Furthermore, the induction of IL-10 immunoregulatory cytokine following SIT has a profound effect on isotype class switching. In the presence of IL-4, additional IL-10 induces a preferential class switch in favour of IgG4 and has been shown to suppress both total and allergen-specific IgE responses [69, 70]. A detailed time-course of IL-10 induction in patients treated with grass pollen-SIT has revealed a significant increase in IL-10 production in parallel with the suppression of late responses and preceding IgG4 induction and clinical responsiveness [17]. Therefore, immunoreactive IgG4 may be a surrogate biomarker of IL-10 induction [17, 71]. Elevated levels of allergen-specific IgA2 antibodies and polymeric IgA2 have been reported following grass pollen-specific injection immunotherapy. Passive sensitization of monocytes *in vitro* using purified polymeric IgA2 from IgA-containing serum obtained following allergen-SIT, followed by cross-linking *in vitro* of IgA on monocytes by antigen or anti-IgA resulted in IL-10 production [39]. This indirect production of IL-10 from accessory cells may in turn favour isotype class switching in favour of IgG4 antibody production. These findings implicate a possible role for IgA antibodies in the induction of tolerance following SIT.

### Sublingual allergen-specific immunotherapy

The clinical efficacy of the sublingual route as an alternative to subcutaneous immunotherapy has been confirmed in Cochrane Systematic Reviews and meta-analyses [72, 73]. Optimal, high doses for grass pollen-SLIT have been established in two large independent randomized double-blind placebo-controlled studies that used grass allergen tablets [74, 75]. A 4- to 6-month pre-seasonal treatment, followed by a continued seasonal treatment with allergen-SLIT resulted in increased clinical efficacy compared with a 2-month pre-seasonal treatment [76]. A recent trial using a rapidly dissolving grass allergen tablet showed a consistent decrease in symptom and rescue medication scores compared with placebo treatment [77] that persisted for 3 years of continued treatment and persisted for at least 1 year following a double-blind cessation of treatment [78].

### Immunological mechanisms of sublingual allergen-specific immunotherapy

The oral mucosa is considered as a site of natural immune tolerance [72]. There are resident professional antigen-presenting cells, namely oral Langerhans cells (oLC), that express high levels of MHC class I and II molecules. Co-stimulatory molecules such as CD40, CD80, CD86 and FcγRI/II are also expressed. oLC constitutively expressed high levels of FcεRI [79] and have been shown to produce IL-10 in a TLR4-dependent manner [80]. Reduced T cell proliferative responses and the induction of T cells with a regulatory phenotype have been demonstrated in co-culture experiments. Cross-linking of FcεRI on monocytes isolated from the oral mucosa induced IL-10 [81] and indoleamine 2,3-dioxygenase [82]. Indoleamine

2,3-dioxygenase is associated with reduced tryptophan levels and impaired T cell effector function. Furthermore, attenuated maturation and a gradual up-regulation of CD83 and CCR7 on oral DCs following allergen uptake during migration to the lymphoid tissue have been demonstrated. During this migration, oral DCs may prime T cells in the oral lymphoid foci before the classical contact with T cells in the lymphoid tissue. DCs residing in the oral mucosa are capable of priming Treg cell, including FoxP3+ Treg cells, which is increased in the oral mucosa during SLIT. IL-10, IL-18 and signalling lymphocytic activation molecule expression and the amount of FoxP3-expressing T cells are increased in the peripheral blood during allergen-SLIT [83, 84]. Moreover, during the pollen season, increased expression of programmed cell death ligand (PD-L1) on B cells and monocytes was associated with reduced IL-4 levels in PBMCs from SLIT-treated patients [85]. Thus, these immunological changes following allergen-SLIT may be associated with the induction of allergen-specific tolerance and the clinical effect of SLIT. These findings suggest a network of Langerhans cells, epithelial cells, monocytes and oral DCs capable of producing IL-10, TGF–β and activins and priming Treg cells [26, 86–88] and may play a potential role in the induction of allergen-specific tolerance and the clinical effect of SLIT. These possibilities require testing pre- and post-allergen-specific sublingual immunotherapy. Additionally, there are limited numbers of mast cells in the oral mucosa, which may possibly account for the established safer profile of SLIT [80] compared with SIT.

The effects of SLIT on allergic Th2 effector cells have been demonstrated in patients treated with *Parietaria*-specific sublingual immunotherapy. A significant reduction in the number of eosinophils, neutrophils and ICAM-1 expression in the nasal mucosa was observed [89]. A reduction of eosinophil cationic protein [89, 90] and numbers of eosinophils have been reported in several but not all studies [91]. The suppression of T cell proliferative response following birch and grass pollen-specific SLIT has been observed [92, 93]. Elevated levels of IFN-γ and/or reduced in Th2 cytokines [90, 94–97] have been reported in some but not all studies [93, 98, 99]. A recent study assessed immunological changes in patients who received pre-seasonal, seasonal or prolonged ragweed sublingual therapy. A reduced expression of CD80 and CD86 expression on CD14+ monocytes, increased IL-10 production and a reduced IL-4 synthesis were observed. Elevated levels of PD-L1 expression and PD-L1-associated IL-10 synthesis were also observed [100]. These findings are in agreement with the previously reported induction of IL-10 at protein and mRNA levels, IgG4 and the modulation of PD-L1, IL-10 and IgG4 [85, 93, 101].

The role of regulatory T cells was elegantly demonstrated following birch pollen SLIT [92]. Higher numbers of circulating CD4+CD25+ T cells were detected after 4 weeks. Elevated levels of FoxP3 and IL-10 were observed in actively treated patients when compared with pre-treatment. IL-4 production and IFN-γ mRNA expression in PBMC cultures were reduced. The loss of suppressor activity was observed when CD25+ cells were depleted from PBMC and was reversed on adding anti-IL-10, which implied the involvement of regulatory T cells. In a blinded trial of SLIT in HDM-sensitive adults, an increased proportion of non-dividing CD4+CD25+FoxP3+ suppressor cells was observed only in mite-treated patients and at 6 and 12 months in 7-day HDM stimulated-PBMC cultures. These phenotypic CD25-associated Treg cells had functional activity in that they were able to suppress mite–antigen-induced CD4+ T cell proliferation and this suppression could be inhibited at 6 months, but not later, by the addition of soluble TGF receptors, implying a transient role for TGF-β in the mechanism of suppression after mite-SLIT. Interestingly, the proportion of these phenotypic Treg cells decreased to baseline levels after 24 months in the actively treated group despite persistent clinical improvement [102]. Serum TGF-β levels have also been shown to correlate with visual analogue scores and rescue medication scores following sublingual pollen-SIT [103]. Taken together, these findings suggest that Treg cells may play a role in reducing Th2-mediated allergic inflammation that is manifest at 3–6 months and IL-10 [92] and/or TGF-β [102] dependent. However, the persistence of antigen-induced suppression and clinical efficacy at later time-points during allergen-SLIT may be mediated by other immunological mechanisms such as immune deviation in favour of antigen-specific Th1 responses [18, 92, 94].

### Immunoglobulin responses following sublingual allergen–specific immunotherapy

During pollen-SLIT, paradoxic marked increases in allergen-specific IgE may occur within weeks, although they do not appear to be associated with adverse events. These early increases are followed by blunting of seasonal increases in serum IgE. There follows an increase in allergen-specific IgG and IgG4. These elevations are both time- and allergen-dose dependent [75] and progressive for at least 2 years [77], although of a lower magnitude than that observed during allergen-SIT [4].

Some studies have shown increases in specific IgG4 in the absence of demonstrable efficacy [93], whereas others have shown no difference in the IgG levels (Table 1), likely related to the lower allergen doses used [98], particularly in relation to HDM-specific sublingual immunotherapy [8, 91, 99, 104]. These findings raise the issue of causality vs. bystander effects of IgG. In functional assays, sera obtained after grass pollen-specific sublingual immunotherapy were able to inhibit IgE-allergen binding *in vitro* [77]. The heterogeneity of immunological responses in particular in relation to allergen-specific IgE and IgG4

**Table 1.** Summary of IgE and IgG4 following sublingual grass pollen-specific immunotherapy (↑, increase; ↓, decrease)

| Studies | Lima et al. [106] | Andre et al. [107] | Bahceciler et al. [92] | Durham et al. [108] | Didier et al. [76] | Amar et al. [109] | Durham et al. [79] | Scadding et al. [65] |
|---|---|---|---|---|---|---|---|---|
| RDBPCT | Yes | No | No | Yes | Yes | Yes | Yes/follow-up | No |
| Subjects | 56 | 110 | 39 | 855 | 628 | 54 | 257 | 24 |
| Allergen | Timothy grass | Ragweed pollen | HDM | Timothy grass | 5 grass | Timothy grass/9 grasses | Timothy grass | Timothy grass |
| Clinical outcome | Primary clinical end points were not significantly different when compared with placebo | Effective in subjects treated with ahigh dose only | No change in daily symptoms | Effective in subjects treated with high dose | ↓ symptoms scores in subjects treated with a high dose compared with a low dose | No change in symptom and rescue medication scores in both treated-groups when compared with placebo | ↓ reductions in symptom scores and medication scores when compared with placebo | Primary clinical end points were not significantly different when compared with placebo |
| Specific IgE | ↑ vs. placebo | ↑ vs. placebo | ↓ in specific IgE in treated groups | Dose-dependent ↑ compared with placebo | Dose-dependent increases vs. placebo | No change | Not measured | ↑ vs. placebo |
| Specific IgG4 | ↑ vs. placebo | ↑ vs. placebo | No change | Dose-dependent↑ compared with placebo | Dose-dependent increases vs. placebo | ↑ in monotherapy-treated group only | Sustained ↑ IgG4 1 year after withdrawal | ↑ IgG4, IgG1, IgA1, IgA2, vs. placebo |
| Inhibition of CD23-dependent IgE-FAB | Not measured | Not measured | Not measured | Not measured | Not measured | Not measured | Not measured (although, Sustained ↑ IgE blocking factor 1 year after withdrawal) | ↑ vs. placebo |

antibodies may be largely explainable on the basis of the dose of allergen used, the duration of treatment and the types of allergens used [105].

In a recent study, we showed that sublingual grass pollen-SLIT induced significant increases in serum-specific IgG1 and IgG4 antibodies levels at 3–18 months that were associated with an increase in IgG-associated serum inhibitory activity for allergen-IgE binding to B cells. Down-regulation of IgE-facilitated binding is a surrogate for the suppression of IgE-facilitated presentation and activation of T cells [59, 61]. A likely consequence is the suppression of otherwise sustained Th2 responses at mucosal surfaces with the consequent suppression of allergic inflammation and associated symptoms on subsequent allergen exposure during the pollen season. Grass pollen-specific IgA1 and IgA2 were increased during the pollen season in SLIT- but not in placebo-treated patients. These data suggest a likely mechanism of action of SLIT similar to that of SCIT. In this study, there were no associated changes in allergen-specific IgG2 and IgG3 antibody levels following SLIT, in contrast to the findings observed in allergen-SIT. This may be due to the different route of allergen administration and, possibly, the presence of professional antigen-presenting cells, such as oral Langerhans cells or DCs in the sub-mucosa. These cells express high levels of high-affinity IgE receptors and when activated following cross linking by allergen produce IL-10 that favours IgG4 class switching by B cells [80]. Moreover, the shift in T cell polarization results in immune deviation from a Th2 towards a Th1 response and a cytokine milieu, which may potentiate IFN-γ production. IFN-γ can promote IgM-producing B cells to class switch to IgG1-producing B cells. Additionally, the increase in *P. pratense*-specific IgA1 and IgA2 in SLIT-treated patients may be the result of enhanced TGF-β expression from antigen-presenting cells or regulatory T cells induced following sublingual allergen-SIT. Although there are similarities between the mechanisms of SLIT and SIT, there are also likely additional local mechanisms involved, as indicated by the observations of Allam et al. [80] and our observations of increased local numbers of FoxP3 expressing CD25+ cells within the sublingual mucosa after SLIT and their increase in numbers during natural pollen exposure following allergen-SLIT [64].

## Immunological mechanisms of long-term benefits of Immunotherapy

A particular feature of allergen-SIT is the induction of long-term antigen-specific tolerance, namely persisting clinical benefit for several years after treatment is withdrawn [6]. These findings, after 3 years of grass pollen-SIT, have now been confirmed in a separate study after 2 years of blinded treatment and withdrawal [65]. Long-term clinical tolerance was associated with the persistence

of IgG-associated inhibitory activity against binding of IgE-allergen complexes to B cells, a surrogate of IgE-facilitated antigen presentation and activation of antigen-specific T cells [65]. In this study, IgG-associated inhibitory bioactivity rather than absolute levels of the IgG4 antibody correlated with clinical tolerance as reflected in maintained suppressed combined symptom and rescue medication scores 2 years after immunotherapy withdrawal. This effect has been demonstrated following grass pollen immunotherapy withdrawal, whereas we could not confirm the maintained suppression of IgE-FAB following the withdrawal of bee venom immunotherapy [110], implying possible different underlying mechanisms for maintained tolerance after the discontinuation of venom immunotherapy.

More recently, a 3-year large randomized double-blind controlled trial of sublingual grass tablet immunotherapy has been shown to provide clinically relevant, sustained disease-modifying effects after a 1-year withdrawal [78]. Throughout the 3-year treatment period, a progressive increase in IgG4 and the IgE-blocking factor (the IgE-blocking factor is the result of a functional assay that measures the amount of IgE actually hindered from binding to allergen to elicit symptoms) in the actively treated group was observed with no change in the placebo. One year after treatment, the differences in change from the baseline values between the groups were still significantly different for both immunoreactive IgG4 and the IgE-blocking factor. These findings strengthen the role of functional inhibitory IgG, in particular IgG1 and IgG4 antibodies, as surrogate biomarkers of persistence of clinical tolerance to grass pollen immunotherapy.

### Potential biomarkers for monitoring allergen-specific immunotherapy

In a trial of grass pollen immunotherapy, the possible predictive value of the ratio of serum specific-IgE/total IgE was evaluated [111]. Patients who received active treatment showed a transient increase, followed by seasonal blunting in specific IgE. The ratio of serum specific-IgE/total IgE correlated with the clinical response to immunotherapy, whereas this was not observed in another study in relation to the clinical response to HDM immunotherapy [112]. Thus, the usefulness of this ratio requires further evaluation. Although there is evidence that SIT is associated with early increases in the concentration of allergen-specific IgG4 blocking antibodies and modest reductions in allergen-specific IgE as determined by ELISAs, these antibody levels did not correlate with clinical efficacy [46, 113, 114]. Despite this lack of correlation, previous reports have demonstrated that fractionated IgG4 antibodies in serum from patients who had received grass pollen immunotherapy were responsible for the inhibition of IgE-FAB to B cells [60, 62]. This suggests a functional role of IgG4 in the inhibition of IgE-FAB, which may be due to changes in IgG antibody specificity or affinity. Another role of allergen-specific IgG4 antibodies induced by immunotherapy is their ability to block allergen-induced IgE-dependent histamine release by basophils, confirming the functional blocking activity of these antibodies [115].

Clinical surrogate markers such as end-point skin prick test (SPT) titration to allergens, airway-specific and non-specific hyperresponsiveness and nasal or conjunctival challenges have been assessed for their potential role in monitoring immunotherapy [116, 117]. In an open non-randomized study in mite-sensitive children, the suppression of immediate skin test reactivity at 3 years protected against relapse and the need for re-treatment in the following 3 years after the discontinuation of immunotherapy [118]. In blinded trials, Walker and colleagues demonstrated complete inhibition of seasonal increases in methacholine-induced BHR, whereas Roberts and colleagues demonstrated significant reductions in allergen-induced cutaneous and conjuctival responses, both following grass pollen immunotherapy. Although there are associations between clinical responses to grass pollen immunotherapy and clinical surrogates such as SPT titration to allergens, allergen-specific bronchial responsiveness and nasal or conjunctival challenges, true correlations with the magnitude of the clinical response in terms of reduced symptoms or improved quality of life have yet to be convincingly demonstrated. This partially reflects the complexity and interdependency of the underlying mechanisms, but also the multiple additional factors involved such as neurogenic influences, target organ responsiveness and, not the least, psychosocial influences of altered environmental factors.

### Mechanisms of peptide-specific immunotherapy

Peripheral T cell tolerance has also been demonstrated following the administration of a synthetic Fel d 1 and Api m 1 peptide-based vaccine. These short peptides consist of a native sequence or a sequence that has undergone amino acid substitution and has reduced IgE cross-linking activity. A mixture of large number of short peptides resulted in the induction of IL-10 and reduced IFN-$\gamma$, IL-4 and IL-13. T cell proliferative responses were also reduced [109]. Inductions of IL-10+ regulatory T cells have also been demonstrated following peptide immunotherapy (PIT) [119]. Isolated CD4+ T cells after PIT in cat-allergic, asthmatic patients actively suppressed the allergen-specific proliferative responses of pre-treatment CD4-PBMCs in co-culture experiments [119]. Furthermore, in a recent study, PIT induced a linked epitope suppression of antigen-specific responses in allergic asthmatic subjects [120]. In a randomized, double-blind placebo-controlled study of PIT consisting of 12 Fel d 1 peptides mixture from

a therapeutic vaccine, PBMC proliferative responses were assessed for each of the 16 Fel d 1 peptides (12 treatment peptides and four Fel d 1 peptides that were not included in the vaccine). Responses to all 12 treatment peptides were significantly reduced in the 16 cat-allergic patients receiving active treatment, but not in the eight subjects receiving placebo [120].

## Concluding comments

Allergen-SIT is effective, antigen-specific and induces long-lasting tolerance. Successful immunotherapy is accompanied by the suppression of allergic inflammation and the induction Treg cells and 'protective and non-inflammatory' antibodies such as IgA2, IgG and particularly IgG4 that inhibit facilitated antigen presentation and basophil histamine release and augment monocyte-derived IL-10 production. The underlying mechanisms involve immune deviation in favour of Th1 responses, induction of FOXP3 and IL-10/TGF-β+Treg in the peripheral blood and in the nasal/oral mucosa. A greater understanding of mechanisms has informed novel immunotherapy approaches [121], whereas the identification of predictive biomarkers for the success of immunotherapy remains elusive.

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## References

1 Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 1998; **102**:558–62.
2 Lockey RF. ''ARIA'': global guidelines and new forms of allergen immunotherapy. *J Allergy Clin Immunol* 2001; **108**:497–99.
3 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**:87–93.
4 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**:319–25.
5 Calderon MA, Alves B, Jacobson M, Hurwitz B, Sheikh A, Durham S. Allergen injection immunotherapy for seasonal allergic rhinitis. *Cochrane Database Syst Rev* 2007: CD001936.
6 Durham SR, Walker SM, Varga EM *et al*. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 1999; **341**:468–75.
7 Golden DB, Kagey-Sobotka A, Norman PS, Hamilton RG, Lichtenstein LM. Outcomes of allergy to insect stings in children, with and without venom immunotherapy. *N Engl J Med* 2004; **351**:668–74.
8 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**:1392–97.
9 Moller C, Dreborg S, Ferdousi HA *et al*. Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). *J Allergy Clin Immunol* 2002; **109**:251–56.
10 Niggemann B, Jacobsen L, Dreborg S *et al*. Five-year follow-up on the PAT study: specific immunotherapy and long-term prevention of asthma in children. *Allergy* 2006; **61**:855–59.
11 Pienkowski MM, Norman PS, Lichtenstein LM. Suppression of late-phase skin reactions by immunotherapy with ragweed extract. *J Allergy Clin Immunol* 1985; **76**:729–34.
12 Iliopoulos O, Proud D, Adkinson NF Jr *et al*. Effects of immunotherapy on the early, late, and rechallenge nasal reaction to provocation with allergen: changes in inflammatory mediators and cells. *J Allergy Clin Immunol* 1991; **87**:855–66.
13 Warner JO, Price JF, Soothill JF, Hey EN. Controlled trial of hyposensitisation to *Dermatophagoides pteronyssinus* in children with asthma. *Lancet* 1978; **2**:912–15.
14 Varney VA, Hamid QA, Gaga M *et al*. Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J Clin Invest* 1993; **92**:644–51.
15 Nish WA, Charlesworth EN, Davis TL *et al*. The effect of immunotherapy on the cutaneous late phase response to antigen. *J Allergy Clin Immunol* 1994; **93**:484–93.
16 Benjaponpitak S, Oro A, Maguire P, Marinkovich V, DeKruyff RH, Umetsu DT. The kinetics of change in cytokine production by CD4 T cells during conventional allergen immunotherapy. *J Allergy Clin Immunol* 1999; **103**:468–75.
17 Francis JN, James LK, Paraskevopoulos G *et al*. Grass pollen immunotherapy: IL-10 induction and suppression of late responses precedes IgG4 inhibitory antibody activity. *J Allergy Clin Immunol* 2008; **121**:1120–25, e2.
18 Puggioni F, Durham SR, Francis JN. Monophosphoryl lipid A (MPL) promotes allergen-induced immune deviation in favour of Th1 responses. *Allergy* 2005; **60**:678–84.
19 Nanda A, O'connor M, Anand M *et al*. Dose dependence and time course of the immunologic response to administration of standardized cat allergen extract. *J Allergy Clin Immunol* 2004; **114**:1339–44.

20 Arvidsson MB, Löwhagen O, Rak S. Allergen-specific immunotherapy attenuates early and late phase reactions in lower airways of birch pollen asthmatic patients: a double blind placebo-controlled study. *Allergy* 2004; **59**:74–80.

21 Jutel M, Akdis M, Budak F *et al*. IL-10 and TGF-β cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003; **33**:1205–14.

22 Faith A, Richards DF, Verhoef A, Lamb JR, Lee TH, Hawrylowicz CM. Impaired secretion of interleukin-4 and interleukin-13 by allergen-specific T cells correlates with defective nuclear expression of NF-AT2 and jun B: relevance to immunotherapy. *Clin Exp Allergy* 2003; **33**:1209–15.

23 Wachholz PA, Nouri-Aria KT, Wilson DR *et al*. Grass pollen immunotherapy for hayfever is associated with increases in local nasal but not peripheral Th1: Th2 cytokine ratios. *Immunology* 2002; **105**:56–62.

24 Francis JN, Till SJ, Durham SR. Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol* 2003; **111**:1255–61.

25 Durham SR, Ying S, Varney VA *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**:1356–65.

26 Akbari O, DeKruyff RH, Umetsu DT. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nat Immunol* 2001; **2**:725–31.

27 Tversky JR, Bieneman AP, Chichester KL, Hamilton RG, Schroeder JT. Subcutaneous allergen immunotherapy restores human dendritic cell innate immune function. *Clin Exp Allergy* 2010; **40**:94–102.

28 Dreschler K, Bratke K, Petermann S *et al*. Impact of immunotherapy on blood dendritic cells in patients with Hymenoptera venom allergy. *J Allergy Clin Immunol* 2011; **127**:487–94, e1–3.

29 de Heer HJ, Hammad H, Soullie T *et al*. Essential role of lung plasmacytoid dendritic cells in preventing asthmatic reactions to harmless inhaled antigen. *J Exp Med* 2004; **200**:89–98.

30 Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. *J Clin Invest* 1998; **102**:98–106.

31 Ling EM, Smith T, Nguyen XD *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**:608–15.

32 Mobs C, Slotosch C, Loffler H, Jakob T, Hertl M, Pfutzner W. Birch pollen immunotherapy leads to differential induction of regulatory T cells and delayed helper T cell immune deviation. *J Immunol* 2010; **184**:2194–203.

33 Gorelik L, Constant S, Flavell RA. Mechanism of transforming growth factor b-induced inhibition of T helper type 1 differentiation. *J Exp Med* 2002; **195**:1499–505.

34 Gorelik L, Fields PE, Flavell RA. Cutting edge: TGF-b inhibits Th type 2 development through inhibition of GATA-3 expression. *J Immunol* 2000; **165**: 4773–477.

35 Karagiannidis C, Akdis M, Holopainen P *et al*. Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma. *J Allergy Clin Immunol* 2004; **114**:1425–33.

36 Chen W, Jin W, Hardegen N *et al*. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-b induction of transcription factor Foxp3. *J Exp Med* 2003; **198**:1875–86.

37 Fantini MC, Becker C, Monteleone G, Pallone F, Galle PR, Neurath MF. Cutting edge: TGF-b induces a regulatory phenotype in CD4+CD25 T cells through Foxp3 induction and down-regulation of Smad7. *J Immunol* 2004; **172**:5149–53.

38 Oida T, Xu L, Weiner HL, Kitani A, Strober W. TGF-β-mediated suppression by CD4+CD25+ T cells is facilitated by CTLA-4 signaling. *J Immunol* 2006; **177**:2331–19.

39 Pilette C, Nouri-Aria KT, Jacobson MR *et al*. Grass pollen immunotherapy induces an allergen-specific IgA2 antibody response associated with mucosal TGF-β expression. *J Immunol* 2007; **178**:4658–66.

40 Grindebacke H, Larsson P, Wing K, Rak S, Rudin A. Specific immunotherapy to birch allergen does not enhance suppression of Th2 cells by CD4(+)CD25(+) regulatory T cells during pollen season. *J Clin Immunol* 2009; **29**:752–60.

41 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, 72 e1.

42 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**:73–79.

43 Wilson DR, Irani AM, Walker SM *et al*. Grass pollen immunotherapy inhibits seasonal increases in basophils and eosinophils in the nasal epithelium. *Clin Exp Allergy* 2001; **31**:1705–13.

44 Tsai YG *et al*. TLR2 agonists enhance CD8+Foxp3+ regulatory T cells and suppress Th2 immune responses during allergen immunotherapy. *J Immunol* 2010; **184**:7229–37.

45 Wilson DR, Nouri-Aria KT, Walker SM *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**:971–76.

46 Van Ree R, Van Leeuwen WA, Dieges PH *et al*. Measurement of IgE antibodies against purified grass pollen allergens (Lol p 1, 2, 3 and 5) during immunotherapy. *Clin Exp Allergy* 1997; **27**: 68–74.

47 Lichtenstein LM, Ishizaka K, Norman PS, Sobotka AK, Hill BM. IgE antibody measurements in ragweed hay fever. Relationship to clinical severity and the results of immunotherapy. *J Clin Invest* 1973; **52**:472–82.

48 Gleich GJ, Zimmermann EM, Henderson LL, Yunginger JW. Effect of immunotherapy on immunoglobulin E and immunoglobulin G antibodies to ragweed antigens: a six-year prospective study. *J Allergy Clin Immunol* 1982; **70**: 261–71.

49 Moverare R, Elfman L, Vesterinen E, Metso T, Haahtela T. Development of new IgE specificities to allergenic components in birch pollen extract during specific immunotherapy studied with immunoblotting and Pharmacia CAP System. *Allergy* 2002; **57**:423–30.

50 Gehlar K, Schlaak M, Becker W-M, Buffe A. Monitoring allergen Immunotherapy

of pollen-allergic patients: The ration of allergen-specificIgG4 to IgG1 correlates with clinical outcome. *Clin Exp Allergy* 1999; **29**:497–506.

51 Moverare R, Vesterinen E, Metso T, Sorva R, Elfman L, Haahtela T. Pollen-specific rush immunotherapy: clinical efficacy and effects on antibody concentrations. *Ann Allergy Asthma Immunol* 2001; **86**:337–42.

52 Devey ME, Wilson DV, Wheeler AW. The IgG subclasses of antibodies to grass pollen allergens produced in hay fever patients during hyposensitization. *Clin Allergy* 1976; **6**:227–36.

53 Mothes N, Heinzkill M, Drachenberg KJ *et al*. Allergen-specific immunotherapy with a monophosphoryl lipid A-adjuvanted vaccine: reduced seasonally boosted immunoglobulin E production and inhibition of basophil histamine release by therapy-induced blocking antibodies. *Clin Exp Allergy* 2003; **33**:1198–208.

54 Garcia BE, Sanz ML, Dieguez I, de las Marinas MD, Oehling A. Modifications in IgG subclasses in the course of immunotherapy with grass pollen. *J Investig Allergol Clin Immunol* 1993; **3**:19–25.

55 Lambin P, Bouzoumou A, Murrieta M *et al*. Purification of human IgG4 subclass with allergen-specific blocking activity. *J Immunol Methods* 1993; **165**:99–111.

56 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**:577–85.

57 Zhu D, Kepley CL, Zhang K, Terada T, Yamada T, Saxon A. A chimeric human–cat fusion protein blocks cat-induced allergy. *Nat Med* 2005; **11**:446–49.

58 Kepley CL, Taghavi S, Mackay G *et al*. Co-aggregation of FcgammaRII with FcepsilonRI on human mast cells inhibits antigen-induced secretion and involves SHIP-Grb2-Dok complexes. *J Biol Chem* 2004; **279**:35139–49.

59 Ejrnaes AM, Svenson M, Lund G, Larsen JN, Jacobi H. Inhibition of rBet v 1-induced basophil histamine release with specific immunotherapy -induced serum immunoglobulin G: no evidence that FcgammaRIIB signalling is important. *Clin Exp Allergy* 2006; **36**:273–82.

60 van Neerven RJ, Wikborg T, Lund G *et al*. Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4+ T cells by inhibiting serum-IgE-facilitated allergen presentation. *J Immunol* 1999; **163**: 2944–52.

61 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**:915–22.

62 Wurtzen PA, Lund G, Lund K, Arvidsson M, Rak S, Ipsen H. A double-blind placebo-controlled birch allergy vaccination study II: correlation between inhibition of IgE binding, histamine release and facilitated allergen presentation. *Clin Exp Allergy* 2008; **38**: 1290–301.

63 Nouri-Aria KT, Wachholz PA, Francis JN *et al*. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J Immunol* 2004; **172**:3252–59.

64 Pree I, Shamji MH, Kimber I, Valenta R, Durham SR, Niederberger V. Inhibition of CD23-dependent facilitated allergen binding to B cells following vaccination with genetically modified hypoallergenic Bet v 1 molecules. *Clin Exp Allergy* 2010; **40**:1346–52.

65 Scadding GW, Shamji MH, Jacobson MR *et al*. Sublingual grass pollen immunotherapy is associated with increases in sublingual Foxp3-expressing cells and elevated allergen-specific immunoglobulin G4, immunoglobulin A and serum inhibitory activity for immunoglobulin E-facilitated allergen binding to B cells. *Clin Exp Allergy* 2010; **40**:598–606.

66 James LK, Shamji MH, Walker SM *et al*. Long-term tolerance following allergen immunotherapy is accompanied by selective persistence of blocking antibodies. *J Allergy Clin Immunol* 2011; **127**:509–16, e1–5.

67 Punnonen J, Aversa G, de Vries JE. Human pre-B cells differentiate into Ig-secreting plasma cells in the presence of interleukin-4 and activated CD4+ T cells or their membranes. *Blood* 1993; **82**:2781–89.

68 Jabara HH, Loh R, Ramesh N, Vercelli D, Geha RS. Sequential switching from mu to epsilon via gamma 4 in human B cells stimulated with IL-4 and hydrocortisone. *J Immunol* 1993; **151**:4528–33.

69 Agresti A, Vercelli D. Analysis of gamma4 germline transcription in human B cells. *Int Arch Allergy Immunol* 1999; **118**:279–81.

70 Meiler F, Klunker S, Zimmermann M, Akdis CA, Akdis M. Distinct regulation of IgE, IgG4 and IgA by T regulatory cells and Toll-like receptors. *Allergy* 2008; **63**:1455–63.

71 Satoguina JS, Weyand E, Larbi J, Hoerauf A. T regulatory-1 cells induce IgG4 production by B cells: role of IL-10. *J Immunol* 2005; **174**:4718–26.

72 Bellinghausen I, Knop J, Saloga J. The role of interleukin 10 in the regulation of allergic immune responses. *Int Arch Allergy Immunol* 2001; **126**:97–101.

73 Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* 2005; **60**:4–12.

74 Radulovic S, Calderon MA, Wilson D, Durham S. Sublingual immunotherapy for allergic rhinitis. *Cochrane Database Syst Rev* 2010; **12**:CD002893.

75 Durham SR, Riis B. Grass allergen tablet immunotherapy relieves individual seasonal eye and nasal symptoms, including nasal blockage. *Allergy* 2007; **62**:954–57.

76 Didier A, Malling HJ, Worm M *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**:1338–45.

77 Calderon MA, Birk AO, Andersen JS, Durham SR. Prolonged preseasonal treatment phase with Grazax sublingual immunotherapy increases clinical efficacy. *Allergy* 2007; **62**:958–61.

78 Dahl R, Kapp A, Colombo G *et al*. Sublingual grass allergen tablet immunotherapy provides sustained clinical benefit with progressive immunologic changes over 2 years. *J Allergy Clin Immunol* 2008; **121**:512–18, e2.

79 Durham SR, Emminger W, Kapp A *et al*. Long-term clinical efficacy in grass pollen-induced rhinoconjunctivitis after treatment with SQ-standardized grass allergy immunotherapy tablet. *J Allergy Clin Immunol* 2010; **125**: 131–38, e1–7.

80 Allam JP, Novak N, Fuchs C *et al*. Characterization of dendritic cells from human oral mucosa: a new Langerhans' cell type with high constitutive FcepsilonRI expression. *J Allergy Clin Immunol* 2003; **112**:141–48.

81 Allam JP, Peng WM, Appel T *et al*. Toll-like receptor 4 ligation enforces tolerogenic properties of oral mucosal Lan-

gerhans cells. *J Allergy Clin Immunol* 2008; **121**:368–74, e1.
82 Novak N, Bieber T, Katoh N. Engagement of Fcepsilon RI on human monocytes induces the production of IL-10 and prevents their differentiation in dendritic cells. *J Immunol* 2001; **167**:797–804.
83 Nieminen K, Laaksonen K, Savolainen J. Three-year follow-up study of allergen- induced in vitro cytokine and signalling lymphocytic activation molecule mRNA responses in peripheral blood mononuclear cells of allergic rhinitis patients undergoing specific immunotherapy. *Int Arch Allergy Immunol* 2009; **150**:370–76.
84 Laaksonen K, Junikka M, Lahesmaa R, Terho EO, Savolainen J. In vitro allergen- induced mRNA expression of signaling lymphocytic activation molecule by PBMC of patients with allergic rhinitis is increased during specific pollen immunotherapy. *J Allergy Clin Immunol* 2003; **112**:1171–77.
85 Piconi S, Trabattoni D, Rainone V *et al*. Immunological effects of sublingual immunotherapy: clinical efficacy is associated with modulation of programmed cell death ligand 1, IL-10, and IgG4. *J Immunol* 2010; **185**:7723–30.
86 von Bubnoff D, Matz H, Frahnert C *et al*. FcepsilonRI induces the tryptophan degradation pathway involved in regulating T cell responses. *J Immunol* 2002; **169**:1810–16.
87 Allam JP, Wurtzen PA, Reinartz M *et al*. Phl p 5 resorption in human oral mucosa leads to dose-dependent and time-dependent allergen binding by oral mucosal Langerhans cells, attenuates their maturation, and enhances their migratory and TGF-β1 and IL-10-producing properties. *J Allergy Clin Immunol* 2010; **126**:638–45, e1.
88 Wakkach A, Fournier N, Brun V, Breittmayer JP, Cottrez F, Groux H. Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation in vivo. *Immunity* 2003; **18**:605–17.
89 Passalacqua G, Albano M, Riccio A *et al*. Clinical and immunologic effects of a rush sublingual immunotherapy to Parietaria species: a double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 1999; **104**:964–68.
90 Passalacqua G, Albano M, Fregonese L *et al*. Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite-induced rhinoconjunctivitis. *Lancet* 1998; **351**:629–32.
91 Ippoliti F, De Santis W, Volterrani A *et al*. Immunomodulation during sublingual therapy in allergic children. *Pediatr Allergy Immunol* 2003; **14**:216–21.
92 Bahceciler NN, Arikan C, Taylor A *et al*. Impact of sublingual immunotherapy on specific antibody levels in asthmatic children allergic to house dust mites. *Int Arch Allergy Immunol* 2005; **136**:287–94.
93 Bohle B, Kinaciyan T, Gerstmayr M, Radakovics 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; **120**:707–13.
94 Fanta C, Bohle B, Hirt W *et al*. Systemic immunological changes induced by administration of grass pollen allergens via the oral mucosa during sublingual immunotherapy. *Int Arch Allergy Immunol* 1999; **120**:218–24.
95 Ebner C, Siemann U, Bohle B *et al*. Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from TH2 to TH1 in T-cell clones specific for Phl p 1, a major grass pollen allergen. *Clin Exp Allergy* 1997; **27**:1007–15.
96 Cosmi L, Santarlasci V, Angeli R *et al*. Sublingual immunotherapy with Dermatophagoides monomeric allergoid down-regulates allergen-specific immunoglobulin E and increases both interferon-gamma- and interleukin-10-production. *Clin Exp Allergy* 2006; **36**:261–72.
97 Savolainen J, Jacobsen L, Valovirta E. Sublingual immunotherapy in children modulates allergen-induced in vitro expression of cytokine mRNA in PBMC. *Allergy* 2006; **61**:1184–90.
98 Savolainen J, Nieminen K, Laaksonen K *et al*. Allergen-induced in vitro expression of IL-18, SLAM and GATA-3 mRNA in PBMC during sublingual immunotherapy. *Allergy* 2007; **62**: 949–53.
99 Rolinck-Werninghaus C, Kopp M, Liebke C, Lange J, Wahn U, Niggemann B. Lack of detectable alterations in immune responses during sublingual immunotherapy in children with seasonal allergic rhinoconjunctivitis to grass pollen. *Int Arch Allergy Immunol* 2005; **136**:134–41.
100 Dehlink E, Eiwegger T, Gerstmayr M *et al*. Absence of systemic immunologic changes during dose build-up phase and early maintenance period in effective specific sublingual immunotherapy in children. *Clin Exp Allergy* 2006; **36**:32–39.
101 Ciprandi G, Fenoglio D, Cirillo I *et al*. Induction of interleukin 10 by sublingual immunotherapy for house dust mites: a preliminary report. *Ann Allergy Asthma Immunol* 2005; **95**:38–44.
102 Ciprandi G, Cirillo I, Fenoglio D, Marseglia G, Tosca MA. Sublingual immunotherapy induces spirometric improvement associated with IL-10 production: preliminary reports. *Int Immunopharmacol* 2006; **6**:1370–73.
103 Ciprandi G, De Amici M, Tosca MA, Pistorio A, Marseglia GL. Sublingual immunotherapy affects specific antibody and TGF-β serum levels in patients with allergic rhinitis. *Int J Immunopathol Pharmacol* 2009; **22**:1089–96.
104 Lue KH, Lin YH, Sun HL, Lu KH, Hsieh JC, Chou MC. Clinical and immunologic effects of sublingual immunotherapy in asthmatic children sensitized to mites: a double-blind, randomized, placebo-controlled study. *Pediatr Allergy Immunol* 2006; **17**:408–15.
105 Tonnel AB, Scherpereel A, Douay B *et al*. Allergic rhinitis due to house dust mites: evaluation of the efficacy of specific sublingual immunotherapy. *Allergy* 2004; **59**:491–97.
106 Andre C, Perrin-Fayolle M, Grosclaude M *et al*. A double-blind placebo-controlled evaluation of sublingual immunotherapy with a standardized ragweed extract in patients with seasonal rhinitis. Evidence for a dose-response relationship. *Int Arch Allergy Immunol* 2003; **131**:111–18.
107 Durham SR, Yang WH, Pedersen MR, Johansen N, Rak S. Sublingual immunotherapy with once-daily grass allergen tablets: a randomized controlled trial in seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2006; **117**:802–09.
108 Amar SM, Harbeck RJ, Sills M, Silveira LJ, O'Brien H, Nelson HS. Response to sublingual immunotherapy with grass pollen extract: monotherapy versus combination in a multiallergen extract. *J Allergy Clin Immunol* 2009; **124**:150–56, e1–5.
109 Oldfield WL, Larche M, Kay AB. Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production

in patients sensitive to cats: a randomised controlled trial. *Lancet* 2002; **360**:47–53.
110 Scadding G, Durham S. Mechanisms of sublingual immunotherapy. *J Asthma* 2009; **46**:322–34.
111 Varga EM, Francis JN, Zach MS, Klunker S, Aberer W, Durham SR. Time course of serum inhibitory activity for facilitated allergen–IgE binding during bee venom immunotherapy in children. *Clin Exp Allergy* 2009; **39**:1353–7.
112 Di Lorenzo G, Mansueto P, Pacor ML *et al.* Evaluation of serum s-IgE/total IgE ratio in predicting clinical response to allergen-specific immunotherapy. *J Allergy Clin Immunol* 2009; **123**:1103–10, 10e1–4.
113 Eifan AO, Akkoc T, Yildiz A *et al.* Clinical efficacy and immunological mechanisms of sublingual and subcutaneous immunotherapy in asthmatic/rhinitis children sensitized to house dust mite: an open randomized controlled trial. *Clin Exp Allergy* 2010; **40**:922–32.
114 Ewan PW, Deighton J, Wilson AB, Lachmann PJ. Venom-specific IgG antibodies in bee and wasp allergy: lack of correlation with protection from stings. *Clin Exp Allergy* 1993; **23**: 647–60.
115 Wachholz PA, Durham SR. Mechanisms of immunotherapy: IgG revisited. *Curr Opin Allergy Clin Immunol* 2004; **4**:313–18.
116 Ball T, Sperr WR, Valent P *et al.* Induction of antibody responses to new B cell epitopes indicates vaccination character of allergen immunotherapy. *Eur J Immunol* 1999; **29**:2026–36.
117 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**:263–68.
118 Creticos PS, Schroeder JT, Hamilton RG *et al.* Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. *N Engl J Med* 2006; **355**:1445–55.
119 Verhoef A, Alexander C, Kay AB, Larche M. T cell epitope immunotherapy induces a CD4+ T cell population with regulatory activity. *PLoS Med* 2005; **2**:e78.
120 Campbell JD, Buckland KF, McMillan SJ *et al.* Peptide immunotherapy in allergic asthma generates IL-10-dependent immunological tolerance associated with linked epitope suppression. *J Exp Med* 2009; **206**:1535–47.
121 Larche M. Immunotherapy: Future. *Clin Exp Allergy* 2011.