

Review Series: Immunotherapy and Tolerance—Cutting Edge

Allergen Immunotherapy and Tolerance

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ABSTRACT

Successful allergen-specific immunotherapy (AIT) is associated with a marked decrease in symptoms on allergen exposure, a reduced requirement for 'rescue' anti-allergic drugs and improvement in patients' quality of life. These benefits persist for at least several years following discontinuation of immunotherapy - the hallmark of clinical and immunological tolerance. AIT has been shown to modulate both innate and adaptive immunological responses. Early suppression of innate effector cells of allergic inflammation (mast cells, basophils), regulation of pro-allergic T helper 2 type (Th 2) responses and IgE+ B cell responses have been shown to occur both in the tissue and in the peripheral blood during AIT. The allergen-tolerant state is associated with local and systemic induction of distinct populations of allergen-specific T regulatory cells including IL-10+ Tregs (Tr1 cells), TGF- β + Tregs and FoxP3+ memory T regs. B cells are switched in favour of producing IgG (particularly IgG4) antibodies and associated blocking activity for IgE-dependent events, including basophil activation and IgE-facilitated allergen binding to B cells. An induction of IL-10+ B regulatory cells and alterations in dendritic cell subsets have also recently been described. These events are followed by the induction of T regulatory cells, suppression of allergen-specific T cell proliferation and immune deviation from Th2 in favour of Th1 responses. Alternative mechanisms of tolerance include apoptosis/deletion of antigen-specific memory Th2 cells and/or a failure of co-stimulation leading to T cell anergy.

KEY WORDS

allergen immunotherapy, allergy, biomarkers, immune tolerance, rhinitis

INTRODUCTION

Allergic rhinitis (AR) is an IgE-mediated chronic inflammatory disease of the lining of the nasal mucosa.¹ AR is the most common allergic disease in Japan, Western Europe and the United States of America. Its prevalence has increased in recent decades and is still on the rise worldwide.¹⁻⁴ It represents a major socio-economic and health burden.⁵ Pharmacotherapy such as anti-histamines and nasal corticosteroids are effective in the majority of patients. Conversely, in a small proportion of patients, symptoms are not adequately controlled despite high doses of medications. In these patients, allergen immunotherapy (AIT) may

be indicated. AIT is currently the only disease modifying treatment for IgE-mediated allergies that is associated with long-term clinical and immunological tolerance. AIT was initially reported in the early 20th century by Leonard Noon. He described his initial observations on effects of pollen-specific injection immunotherapy resulting in suppression of allergen-induced ocular symptoms.⁶ AIT is conventionally administered by the subcutaneous route (Subcutaneous immunotherapy: SCIT).¹ It has proven efficacy in adults and children who have allergy to house dust mite, animal danders and pollen-induced allergic rhinitis with/without asthma.¹ In recent years, sublingual immunotherapy (SLIT) has been shown to be ef-

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fective and has a superior safety profile compared to SCIT. The efficacy of both SCIT and SLIT for seasonal and perennial allergic rhinitis has been confirmed in recent systematic meta-analyses.⁷⁻¹⁰ In this article, we briefly review evidence for efficacy and present an overview of the immunological mechanisms associated with tolerance induction during and after discontinuation of AIT.

CLINICAL BENEFITS OF AIT

AIT is effective and is current standard practice, especially in those allergic patients who do not respond to pharmacotherapy.¹¹⁻¹³ The efficacy of AIT has been validated using several allergens including grass and tree pollen,^{14,15} house dust mite,¹⁶ insect venom¹⁷ and animal dander.¹⁸ AIT administered either as SCIT or SLIT confers long-term clinical benefit for at least several years after discontinuation of treatment.^{11,19-21} In pollen-induced hayfever, SCIT showed greater clinical effect size in reducing nasal and ocular symptom scores when compared to antihistamines.²² A comprehensive systematic review of the clinical efficacy of SCIT in patients with seasonal allergic rhinitis in collaboration with the Cochrane database evaluated fifty one randomised controlled trials with 2871 participants.⁸ A significant reduction of standardized mean difference in symptoms versus placebo was observed (SMD: -0.73, 95% Confidence Intervals (CI): 0.97 to -0.50, $p < 0.00001$). Assessment of requirement of rescue medication scores in 13 studies revealed a significant reduction in usage of medication intake (SMD: -0.57, 95% CI: -0.82 to -0.33, $p < 0.00001$) in actively treated subjects. SCIT reduced the onset of new sensitisations in children²³ and there is evidence that SCIT may prevent the progression of allergic rhinitis to asthma.²¹ Specific immunotherapy has long-term preventive effects on seasonal and perennial asthma as shown in the 10-year preventive allergy treatment study (PAT study).²¹ Clinical responsiveness to SCIT has been shown to exceed the duration of treatment by several years, a clear advantage over the use of anti-IgE or anti-allergic drugs.^{11,24} A recent updated Cochrane systemic review and meta-analysis focussed on the use of sublingual immunotherapy (SLIT) in allergic rhinitis, including both seasonal and perennial disease and in adult and paediatric populations. There was a significant reduction in total symptom scores (SMD; -0.49, 95% CI -0.64 to -0.34, $P < 0.001$) and rescue medication (SMD; -0.32, 95% CI -0.43, -0.21, $p < 0.001$) compared to placebo-treated participants.²⁵ To date, two randomised placebo controlled trials of SLIT for Japanese cedar pollinosis have been reported.^{26,27} SLIT-treated patients exhibited significantly lower symptom scores compared to placebo and furthermore SLIT for Japanese cedar pollinosis significantly improved rhinitis quality of life scores.

IMMUNOLOGIC MECHANISMS AND TOLERANCE INDUCTION BY AIT

AIT provides a useful human model for studying the induction of clinical and immunological tolerance. Immunologic tolerance induced by allergen immunotherapy involves several phases. The allergen-induced early and late-phase response in the skin and nose may be used as a clinical surrogate to monitor tolerance. An early event is the suppression of the allergen-induced late cutaneous responses within 2-4 weeks of commencing immunotherapy and at low doses of allergen immunotherapy before onset of clinical efficacy. In contrast, the IgE-mediated immediate response in the skin is only partially suppressed by immunotherapy and with a more prolonged time course that becomes evident at 8-16 weeks that corresponds temporally with an increase in IgG antibodies and increases in serum IgG-associated inhibitory activity for both IgE-dependent basophil activation and IgE-facilitated binding of allergen-IgE complexes to B cells²⁸ (Fig. 1). There is also an early desensitisation of basophil effector cell function as reflected by a decrease in allergen-stimulated surface expression of CD63.²⁹

There is an early induction of IL-10 and TGF- β producing T regulatory (Treg) T cells, likely preceded by activation of so-called 'protolerogenic' dendritic cells.³⁰ There follows down regulation of allergen-specific T cell responses and a delayed shift in the T helper type 2 phenotype in favour of Th1 responses. Changes in serum antibodies occur in parallel. These include the induction of 'protective' allergen-specific IgG1, IgG4 and IgA2 antibodies. There is an early paradoxical increase in specific IgE antibodies accompanied by distinct blunting of seasonal pollen-induced increases in specific IgE. There follows a delayed-in-time gradual reduction in allergen-specific IgE to the sensitising allergen over several years during and after withdrawal of immunotherapy.

AIT MODULATE DENDRITIC CELLS TO INDUCE REGULATORY T CELLS

In vivo murine and *in vitro* human studies have shown that pro-inflammatory epithelial derived mediators and cytokines such as TSLP, IL-25, IL-33 prime DCs to polarize naïve T cell responses towards a pro-allergic Th2 phenotype.³¹⁻³⁵ AIT may dampen these inflammatory epithelial responses resulting in induction of tolerogenic DCs which are able to polarize T cells towards an IL-10 producing Treg phenotype. The inducible IL-10+ Tregs may in turn suppress pro-inflammatory DCs and modulate Th2 responses.³⁵⁻³⁷

AIT has been shown to augment peripheral DC TLR9-mediated innate immune function.³⁸ A 3-5-fold increase in IFN- α production by plasmacytoid dendritic cells (pDCs) in response to CpG stimulation *in vitro* was demonstrated in subjects who received

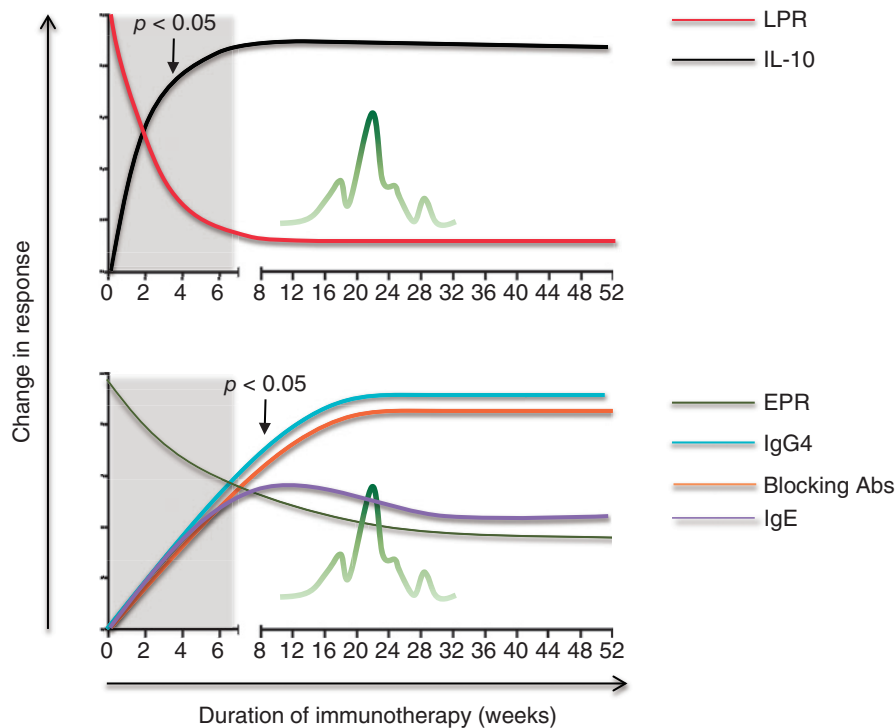


Fig. 1 Time course of response to subcutaneous immunotherapy. Suppression of the late allergic response (LPR) at weeks 2-4 is associated with an increase in IL-10 production. Inhibition of the early allergic responses (EPR) is observed at weeks 8-16 and parallels the induction of immunoreactive IgG4 antibodies and serum IgG-associated 'blocking antibodies'. Shaded grey area represents the up-dosing phase of subcutaneous immunotherapy (cluster regimen). The green line indicates the seasonal increase in grass pollen counts. Reproduced with permission modified from *Clin Exp Allergy* 2008; 38: 1074-88 (ref. 28). For original supporting data, see ref. 15.

HDM specific AIT. A recent study revealed that different subsets of human DCs enriched from peripheral blood could preferentially induce IL-10+ regulatory T cells and subsequently suppressed *in vitro* allergen-driven Th2 responses.³⁰

A novel inhibitory cytokine namely IL-27, which is produced by dendritic cells following TLR4 stimulation by LPS *in vitro*, has been shown to suppress T helper 2 responses in patients with seasonal allergic rhinitis.³⁹ Interleukin (IL)-27 is a heterodimeric cytokine that belongs to the IL-12 family and consists of Epstein Bar inducible gene 3 (EBI-3)⁴⁰ and IL-12p28⁴¹ (ref) (Fig. 2). IL-27 was shown to suppress grass pollen allergen-stimulated PBMC proliferation in a dose-dependent manner, whereas mRNA expression for T-bet and c-Maf was upregulated and GATA-3 was downregulated. IL-27 significantly down-regulated IL-4, IL-5 and up-regulated IL-10 and IFN- γ mRNA expression. IL-27 inhibited IL-4 protein production from Th2 clones when stimulated with anti-CD3/CD28. Moreover, T effector cell proliferation was suppressed when grass pollen-stimulated IL-27-primed DCs were cultured with T effector cells. Although

these findings identify inhibitory effects of IL-27 on Th2 helper responses, its immunomodulatory role during AIT remains to be determined. In another study, proteomic analysis and mass spectroscopy of peripheral human DCs identified 2 potential candidate proteins, namely stabilin 1 (STAB1) and the complement component C1Q as potentially representing a tolerogenic signature of DCs. These proteins may be relevant for inducing tolerogenic T regulatory responses. For example *ex vivo* studies involving quantitative polymerase chain reaction of peripheral blood mononuclear cells purified from blood drawn before/after grass pollen SLIT revealed elevations in STAB1 and C1Q RNA expression that correlated with the clinical response to immunotherapy.³⁰

INDUCTION OF T REGULATORY CELLS BY AIT

Studies have highlighted the role of allergen-specific Tregs in tolerance induction during AIT. The regulatory properties of IL-10+ (Tr1) and TGF- β + inducible Tregs and FoxP3+ natural Tregs (nTregs) have several features in common. IL-10 and TGF- β produced by CD4+CD25+ cells have been reported to modulate

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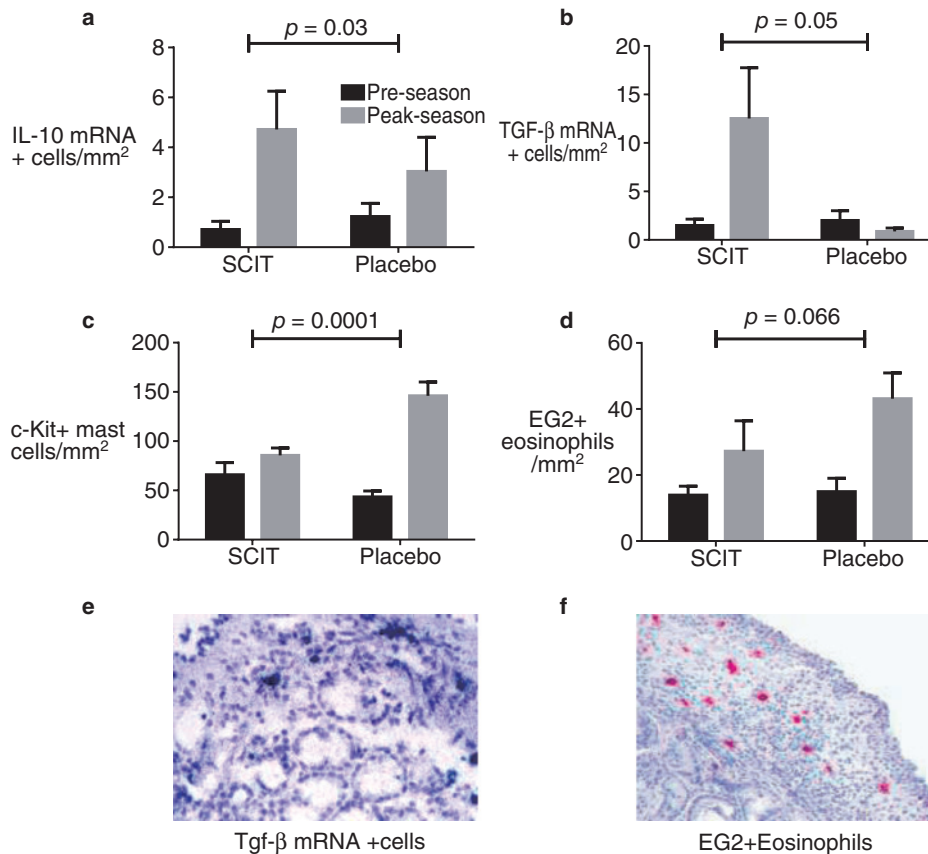


Fig. 3 Effects of 2 years grass pollen subcutaneous immunotherapy on seasonal changes in the nasal mucosa during the pollen season. By *in situ* hybridisation there were significant increases in cells expressing mRNA for the regulatory cytokines IL-10 and TGF- β during the pollen season after immunotherapy when compared to placebo-treated patients - panels **a**) and **b**). These changes paralleled significant inhibition of seasonal increases in mast cells (c-kit+ cells) and eosinophils (EG2+ cells) by immunostaining - **c**) and **d**). Examples of *in situ* hybridisation (for TGF- β) and immunostaining (for EG2+ cells) are shown - **e**) and **f**). (Data obtained from references 55-58).

depend, at least in part, on mechanisms involving direct cell-cell contact.^{48,49} Functional roles have been proposed for membrane CTLA-4,⁴⁹ surface-bound TGF- β ⁴⁹ and the glucocorticoid-induced TNF receptor (GITR).⁴⁵ nTregs modulate allergen-specific T cell responses in healthy, non-atopic individuals. Increased functional regulatory T cell activity and as mentioned above, elevated levels of IL-10 from allergen-stimulated peripheral blood mononuclear cells (PBMC) cultures have been demonstrated within 2-4 weeks of commencing immunotherapy.¹⁵ Allergen specific Tr1 cells have also been reported following birch pollen AIT.⁴² In the same study, enriched peripheral Tr1 cells inhibited allergen-driven proliferative responses. This suppression was inhibited by neutralising IL-10.⁴²

T regulatory cells may produce TGF- β , a cytokine with potent immunomodulatory properties. Thus TGF- β inhibits differentiation of Th1 and Th2 cells by

down regulating the expression of T-bet and GATA-3.^{50,51} TGF- β induces the expression of FoxP3 and stimulates the development of nTregs.⁵² It promotes the expression of CTLA-4 on Treg cells.^{53,54} In addition TGF- β favours B cells to class switch and produce IgA antibodies a recently described feature of allergen immunotherapy.⁵⁵ Furthermore, downregulation of IgE and Fc ϵ RI expression on Langerhans cells (LCs) has been reported. These findings support a role for TGF- β in maintaining and inducing peripheral tolerance during AIT.

Successful subcutaneous pollen immunotherapy has been associated with increased numbers of Tr1 and nTregs in the nasal mucosa.⁵⁵⁻⁵⁷ These increases in Treg cells within the nasal mucosa were associated with reduced numbers of local c-kit-positive mast cells⁵⁸ and eosinophils⁵⁹ (Fig. 3) and with clinical improvement.⁵⁹ Increased numbers of granzyme B+ and IL-10+ CD8+ Foxp3+ Treg cells were also shown in

healthy control subjects and from house dust mite-sensitive asthmatics before and 6 and 12 months after treatment. Pam3CSK4 (a synthetic TLR2 ligand) and Der p 2 co-stimulation expanded the CD8+CD25+Foxp3+ Treg population and inhibited HDM-induced IL-4 production in PBMCs.⁶⁰ IL-10 production by T cells has been consistently shown in several studies.^{15,41,61} IL-10 and IL-4 synergistically acts on B cells to favour B cell class switch towards IgG4 antibody.⁶² In addition, IL-10 has numerous anti-inflammatory characteristics that include inhibition of mast cell activation by IgE,⁶³ inhibition of the production of Th2 cytokines, including IL-5,⁴⁴ and induction of T cell hyporesponsiveness through IL-10 receptor-dependent blockade of CD28 phosphorylation.⁶⁴ The latter is an essential co-stimulatory pathway for T cells during allergen-induced activation by APCs. Alternative mechanisms of tolerance during allergen-specific immunotherapy might include selective apoptosis/deletion of antigen-specific Th2 cell responses⁶⁵ and/or a failure of co-stimulation leading to T cell anergy.⁶⁶

IL-10+ B CELLS (B REGULATORY CELLS)

Several studies have reported that murine B cells can produce IL-10.^{67,68} These cells are phenotypically characterized as CD1dhiCD5+CD19hi and are called B10 cells.⁶⁷ B10 cells have been shown to suppress T cell-dependent inflammatory responses.^{67,68} Adoptive transfer of CD1dhiCD5+ B cells from oxazolone-sensitized mice inhibited contact sensitivity reactions to oxazolone in recipient mice in a IL-10-dependent and antigen-specific manner.⁶⁷ In another study, B cells induced by *Schistosoma mansoni* protected against anaphylaxis and allergic airway inflammation in an IL-10-dependent manner. IL-10+ Bregs suppressed Th2 cells and induced Treg cells, which further inhibited the Th2 allergic inflammatory responses.⁶⁹ In humans, B10 cells have been identified within the CD24hiCD27+ or CD19CD24hiCD38hi B cell compartment.⁷⁰ Moreover, higher proportion of IL-10-producing CD5+ peripheral blood B cells were observed in healthy controls in response to the milk antigen casein when compared with subjects with cow's milk allergy.⁷¹ These findings suggest that Breg cells may be involved in the maintenance of tolerance to allergens. In a recent study,⁷² human IL-10 producing B regulatory cells were identified and shown to express the phenotypic markers CD25 and CD71 together with low expression of CD73. In studies of peripheral blood from patients with bee venom allergy enriched CD73lowCD25hiCD71hi B cells produced high concentrations of IL-10 and inhibited bee venom phospholipase-A (PLA)-specific CD4 T-cell proliferation.⁷² PLA-specific B cells isolated from non-allergic beekeepers showed increased expression of IL-10 and IgG4. Moreover, in a limited number of subjects, the proportion of IL-10 producing PLA-specific B cells was increased following bee venom-

specific immunotherapy.⁷² It is likely that the observed anti-inflammatory IgG4 responses observed following AIT is mediated by IL-10 produced from a variety of cells, including T cells, B cells and also accessory cells such as monocytes and/or dendritic cells.

IMMUNE DEVIATION FROM Th2 TO Th1 RESPONSES

A shift in the ratio of Th1 and Th2 cytokines has been reported in target organs.^{73,74} Horiguchi and colleagues demonstrated an increase in allergen specific Th2 clones after the Japanese cedar pollen season. SLIT using Japanese cedar pollen extract was associated with a decrease in the frequency of Th2 clones induced by pollen exposure.²⁶ Several studies have reported that the induction of long-term clinical tolerance during AIT is associated with a delayed immune deviation of allergen-specific Th2 to Th1 responses.⁷⁵ However, not all *in vitro* studies of peripheral blood T cells from subjects following AIT have demonstrated reductions in allergen-driven proliferative and Th2 cytokine responses.⁷⁶ Several reasons may account for these discrepancies such as variations in laboratory methodology and lack of standardization of allergen extracts used for immunotherapy.

AIT has been shown to modify the cytokine profile of T cells recruited into the target organ.⁷⁶ Nasal mucosal biopsies from AIT treated patients, performed during the allergen-induced late allergic response to grass pollen, revealed increases in IFN- γ mRNA expressing cells⁷⁴ and both increases in IFN- γ and decreases in IL-4 protein levels in nasal fluid.⁷⁷ Interestingly, increases in the numbers of IFN- γ mRNA+ cells in the nasal mucosa that accompanied suppression of the late response after allergen challenge outside the pollen season inversely correlated with clinical symptoms during the pollen season.⁷⁴ In AIT treated patients, there was also a clear association between the suppression of the late cutaneous allergic responses and enhanced IL-12 mRNA expression in the skin. The latter correlated directly with increased IFN- γ and inversely with IL-4 expression.⁷⁸ The principle cell source of IL-12 was found to be macrophages, whereas at the time, specific probes for dendritic cell subsets were unavailable for probing the dendritic cell as an alternative source of IL-12. Similar studies demonstrated that AIT inhibited seasonal increases in IL-5 and IL-9 mRNA expressing cells in the nasal mucosa.^{58,59} Eosinophil numbers in the nasal mucosa after grass pollen immunotherapy correlated directly with IL-5 expression and inversely with clinical symptoms during the pollen season i.e suppression of eosinophils correlated with clinical improvement.⁵⁹ These studies accentuate the relevance of studying "target organ" immune responses rather than the peripheral blood, particularly for diseases induced by inhalant allergens.

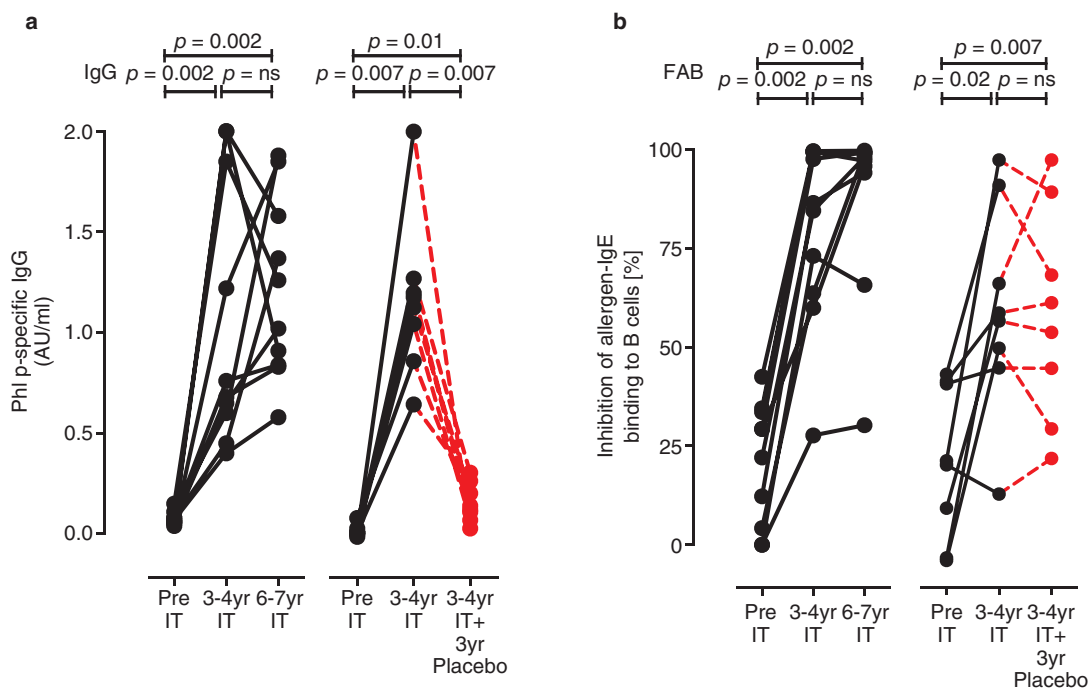


Fig. 4 Persistence of allergen-specific IgG associated inhibitory antibodies after discontinuation of AIT. **(a)** Steady increase in *P. pratense*-specific IgG antibodies in the AIT-treated group (who received 6-7 yr AIT) and a marked decrease in the AIT withdrawn group (who received 3-4 yr AIT followed by placebo injections for 3 years). **(b)** In contrast there is persistence of serum inhibitory activity for IgE-facilitated allergen binding (FAB) following withdrawal of AIT despite an 80% reduction in Specific IgG levels (adapted with permission from James LK *et al. J Allergy Clin Immunol* 2011; 127: 509-516).

IgG4 AND TOLERANCE INDUCTION DURING AND AFTER AIT

Increases in serum IgG and IgG4 antibodies to the sensitizing allergen during successful AIT has been well reported in several studies.⁷⁹⁻⁸⁶ These antibodies have been shown to have inhibitory properties and were first described by Cooke and colleagues in 1935.⁸⁶ Later, Lichtenstein and colleagues described these inhibitory antibodies to be confined to the IgG fraction in serum.⁸⁷ IgA antibodies have also shown to have inhibitory properties.⁸⁸ These inhibitory antibodies or 'blocking antibodies' are thought to compete with IgE for allergen binding to CD23 on the surface of B cells.^{89,90} This suppression of allergen-IgE-binding to B cells (IgE-facilitated binding, IgE-FAB) correlated closely with the observed subsequent inhibition of allergen-induced T-cell proliferation^{29,91,92} (following IgE-facilitated allergen presentation to T cells IgE-FAP). This inhibitory activity for IgE-facilitated allergen binding to B cells that was present in post-immunotherapy serum was shown by affinity chromatography to co-purify largely with IgG4.^{56,92} Measurement of IgE-facilitated allergen-IgE complexes to B cells (IgE-FAB) and its inhibition by post-immunotherapy IgG-containing sera was validated according to Helsinki guidelines as a functional

assay for testing IgG inhibitory activity in sera in large-scale immunotherapy trials.⁹¹ By use of this assay, increases in serum inhibitory activity for IgE-FAB was shown to be immunotherapy dose-dependent and time-dependent, peaking at 3-6 months after commencement of immunotherapy and correlated more closely with suppression of seasonal symptoms when compared with measurement of immunoreactive levels of allergen-specific IgG4.⁷⁹

More recently, Scadding and colleagues have shown that grass pollen sublingual immunotherapy is also associated with increases in antigen-specific IgG1 and IgG4.⁸² In parallel experiments in the same participants there was a time-dependent increase in serum inhibitory activity for IgE-FAB.⁸² Horiguchi and colleague have demonstrated increases in allergen-specific IgG4 after sublingual immunotherapy for Japanese cedar pollen allergy.²⁶

Long-term clinical tolerance after discontinuation of subcutaneous immunotherapy is a cardinal feature of immunotherapy that distinguishes it from treatment with anti-allergic drugs.¹¹ Long-term tolerance after subcutaneous immunotherapy is accompanied by persistent elevations in serum IgG-associated inhibitory activity for IgE-FAB that persists for at least 3 years after discontinuation of treatment (Fig. 4).^{11,81} In this study, IgG-associated inhibitory activity, rather

than absolute levels of IgG4 antibodies, correlated with clinical efficacy following withdrawal of treatment. In patients with a history of insect venom anaphylaxis who underwent subcutaneous bee venom immunotherapy, increases in serum allergen-specific IgG and associated inhibitory activity for IgE-FAB during treatment were not maintained following discontinuation of immunotherapy, implying that different mechanisms may underlie the long-term tolerance observed after discontinuation of venom immunotherapy.^{90,93}

Recent data has confirmed that grass pollen sublingual tablet immunotherapy is also associated with long-term clinical remission.⁹⁴ In a double blind trial of 3 years treatment with grass allergen tablets followed by 2 years follow up, participants who had received active treatment showed sustained and persistent clinical improvement for 2 years after withdrawal of treatment that was accompanied by persistent increases in serum IgG4 and associated inhibitory activity for IgE-FAB whereas this was not observed in placebo-treated subjects.⁹⁴ Taken together these studies support that measurement of serum inhibitory activity for IgE-FAB has potential as a surrogate marker of clinical efficacy and tolerance, whereas it remains to be tested whether IgE-FAB inhibition is able to predict responsiveness to immunotherapy in individual subjects.

CONCLUSION

Allergen immunotherapy is effective and induces long-lasting immunological and clinical tolerance that persists for years following cessation of treatment. Immunotherapy is associated with suppression of allergic inflammation in target organs and increases in IgG4 and IgA2-associated blocking antibodies. The induction of blocking antibodies is accompanied by suppression of undesired allergen-specific Th2 cell responses. This suppression occurs within weeks or months as a result of the induction of regulatory T cells that exert their effects by mechanisms involving cell-cell contact, and also by release of immunomodulatory cytokines such as IL-10 and TGF- β . The more delayed-in-time appearance of antigen-specific Th1 responses and alternative mechanisms such as Th2-cell anergy and/or apoptosis may also be involved. A greater understanding of mechanisms has provided potential surrogate clinical/immunological biomarkers of efficacy and has led to novel immunotherapy strategies, whereas the mechanisms of long-term clinical tolerance remains yet to be further fully elucidated.

REFERENCES

1. Bousquet J, Demoly P, Michel FB. Specific immunotherapy in rhinitis and asthma. *Ann Allergy Asthma Immunol* 2001;**87**(Suppl 1):38-42.
2. Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. *Eur Respir J* 2004;**24**:758-64.
3. Bauchau V, Durham SR. Epidemiological characterization of the intermittent and persistent types of allergic rhinitis. *Allergy* 2005;**60**:350-3.
4. Gotoh M, Yuta A, Okano M, Ohta N, Matsubara A, Okubo K. Severity assessment of Japanese cedar pollinosis using the practical guideline for the management of allergic rhinitis in Japan and the allergic rhinitis and its impact on asthma guideline. *Allergol Int* 2013;**62**:181-9.
5. Reed SD, Lee TA, McCrory DC. The economic burden of allergic rhinitis: a critical evaluation of the literature. *Pharmacoeconomics* 2004;**22**:345-61.
6. Noon L. Prophylactic inoculation against hay fever. *Lancet* 1911;**1**:1572-3.
7. Abramson MJ, Puy RM, Weiner JM. Injection allergen immunotherapy for asthma. *Cochrane Database Syst Rev* 2010;(8):CD001186.
8. 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.
9. Radulovic S, Calderon MA, Wilson D, Durham S. Sublingual immunotherapy for allergic rhinitis. *Cochrane Database Syst Rev* 2010;(12):CD002893.
10. Compalati E, Passalacqua G, Bonini M, Canonica GW. The efficacy of sublingual immunotherapy for house dust mites respiratory allergy: results of a GA2LEN meta-analysis. *Allergy* 2009;**64**:1570-9.
11. Durham SR, Walker SM, Varga EM *et al*. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 1999;**341**:468-75.
12. Bousquet J, Lockey R, Malling HJ *et al*. Allergen immunotherapy: therapeutic vaccines for allergic diseases. World Health Organization. American Academy of Allergy, Asthma and Immunology. *Ann Allergy Asthma Immunol* 1998;**81** (Pt 1):401-5.
13. Bonifazi F, Jutel M, Biló BM, Birnbaum J, Müller U, EAACI Interest Group on Insect Venom Hypersensitivity. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. *Allergy* 2005;**60**:1459-70.
14. Frew AJ, Powell RJ, Corrigan CJ, Durham SR, UK Immunotherapy Study Group. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2006;**117**:319-25.
15. 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**:1120-2.
16. Haugaard L, Dahl R, Jacobsen L. A controlled dose-response study of immunotherapy with standardized, partially purified extract of house dust mite: clinical efficacy and side effects. *J Allergy Clin Immunol* 1993;**91**:709-22.
17. Hunt KJ, Valentine MD, Sobotka AK, Benton AW, Amodio FJ, Lichtenstein LM. A controlled trial of immunotherapy in insect hypersensitivity. *N Engl J Med* 1978;**299**:157-61.
18. Varney VA, Edwards J, Tabbah K, Brewster H, Mavroleon G, Frew AJ. Clinical efficacy of specific immunotherapy to cat dander: a double-blind placebo-controlled trial. *Clin Exp Allergy* 1997;**27**:860-7.
19. Didier A, Worm M, Horak F *et al*. Sustained 3-year efficacy of pre- and coseasonal 5-grass-pollen sublingual immunotherapy tablets in patients with grass pollen-induced rhinoconjunctivitis. *J Allergy Clin Immunol* 2011;**128**:559-66.

20. Marogna M, Spadolini I, Massolo A, Canonica GW, Passalacqua G. Long-lasting effects of sublingual immunotherapy according to its duration: a 15-year prospective study. *J Allergy Clin Immunol* 2010;**126**:969-75.
21. Jacobsen L, Niggemann B, Dreborg S *et al*, The PAT investigator group. Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study. *Allergy* 2007;**62**:943-8.
22. Matricardi PM, Kuna P, Panetta V, Wahn U, Narkus A. Subcutaneous immunotherapy and pharmacotherapy in seasonal allergic rhinitis: a comparison based on meta-analyses. *J Allergy Clin Immunol* 2011;**128**:791-6.
23. 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**:1392-7.
24. 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.
25. Radulovic S, Wilson D, Calderon M, Durham S. Systematic reviews of sublingual immunotherapy (SLIT). *Allergy* 2011;**66**:740-52.
26. Horiguchi S, Okamoto Y, Yonekura S *et al*. A randomized controlled trial of sublingual immunotherapy for Japanese cedar pollinosis. *Int Arch Allergy Immunol* 2008;**146**:76-84.
27. Okubo K, Gotoh M, Fujieda S *et al*. A randomized double-blind comparative study of sublingual immunotherapy for cedar pollinosis. *Allergol Int* 2008;**57**:265-75.
28. James LK, Durham SR. Update on mechanisms of allergen injection immunotherapy. *Clin Exp Allergy* 2008;**38**:1074-88.
29. Würtzen 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.
30. Zimmer A, Bouley J, Le Mignon M *et al*. A regulatory dendritic cell signature correlates with the clinical efficacy of allergen-specific sublingual immunotherapy. *J Allergy Clin Immunol* 2012;**129**:1020-30.
31. Soumelis V, Reche PA, Kanzler H *et al*. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002;**3**:673-80.
32. Ito T, Wang Y-H, Duramad O *et al*. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med* 2005;**202**:1213-23.
33. Hurst SD, Muchamuel T, Gorman DM *et al*. New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *J Immunol* 2002;**169**:443-53.
34. Schmitz J, Owyang A, Oldham E *et al*. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005;**23**:479-90.
35. Bellinghausen I, Brand U, Steinbrink K, Enk AH, Knop J, Saloga J. Inhibition of human allergic T-cell responses by IL-10-treated dendritic cells: differences from hydrocortisone-treated dendritic cells. *J Allergy Clin Immunol* 2001;**108**:242-9.
36. Koya T, Matsuda H, Takeda K *et al*. IL-10-treated dendritic cells decrease airway hyperresponsiveness and airway inflammation in mice. *J Allergy Clin Immunol* 2007;**119**:1241-50.
37. Li X, Yang A, Huang H *et al*. Induction of type 2 T helper cell allergen tolerance by IL-10-differentiated regulatory dendritic cells. *Am J Respir Cell Mol Biol* 2010;**42**:190-9.
38. 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.
39. Matsuoka T, Wing Chun F, Stott B, Durham SR, Shamji MH. Immunomodulatory effects of IL-27 on allergen-induced Th2 responses. *J Allergy Clin Immunol* 2013;**131** (Suppl):AB203.
40. Rousseau F, Basset L, Froger J, Dinguirard N, Chevalier S, Gascan H. IL-27 structural analysis demonstrates similarities with ciliary neurotrophic factor (CNTF) and leads to the identification of antagonistic variants. *Proc Natl Acad Sci U S A* 2010;**107**:19420-5.
41. Pflanz S, Timans JC, Cheung J *et al*. IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4(+) T cells. *Immunity* 2002;**16**:779-90.
42. Bohle B, Kinaciyan T, Gerstmayr M, Radakovic 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.
43. O'Hehir RE, Gardner LM, de Leon MP *et al*. House dust mite sublingual immunotherapy: the role for transforming growth factor-beta and functional regulatory T cells. *Am J Respir Crit Care Med* 2009;**180**:936-47.
44. 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.
45. 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.
46. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995;**155**:1151-64.
47. Swamy RS, Reshamwala N, Hunter T *et al*. Epigenetic modifications and improved regulatory T-cell function in subjects undergoing dual sublingual immunotherapy. *J Allergy Clin Immunol* 2012;**130**:215-7.
48. Sakaguchi S, Sakaguchi N. Regulatory T cells in immunologic self-tolerance and autoimmune disease. *Int Rev Immunol* 2005;**24**:211-26.
49. Nakamura K, Kitani A, Strober W. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. *J Exp Med* 2001;**194**:629-44.
50. Gorelik L, Constant S, Flavell RA. Mechanism of transforming growth factor beta-induced inhibition of T helper type 1 differentiation. *J Exp Med* 2002;**195**:1499-505.
51. Gorelik L, Fields PE, Flavell RA. Cutting edge: TGF-beta inhibits Th type 2 development through inhibition of GATA-3 expression. *J Immunol* 2000;**165**:4773-7.
52. Chen W, Jin W, Hardegen N *et al*. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J Exp Med* 2003;**198**:1875-86.
53. Fantini MC, Becker C, Monteleone G, Pallone F, Galle PR, Neurath MF. Cutting edge: TGF-beta induces a regulatory phenotype in CD4+CD25- T cells through Foxp3 in-

- duction and down-regulation of Smad7. *J Immunol* 2004; **172**:5149-53.
54. Oida T, Xu L, Weiner HL, Kitani A, Strober W. TGF-beta-mediated suppression by CD4+CD25+ T cells is facilitated by CTLA-4 signaling. *J Immunol* 2006; **177**:2331-9.
 55. Pilette C, Nouri-Aria KT, Jacobson MR *et al*. Grass pollen immunotherapy induces an allergen-specific IgA2 antibody response associated with mucosal TGF-beta expression. *J Immunol* 2007; **178**:4658-66.
 56. 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**:3252-9.
 57. 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, 1472.e1.
 58. 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**:73-9.
 59. 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**:971-6.
 60. Tsai Y-G, Yang KD, Niu D-M, Chien J-W, Lin C-Y. TLR2 agonists enhance CD8+Foxp3+ regulatory T cells and suppress Th2 immune responses during allergen immunotherapy. *J Immunol* 2010; **184**:7229-37.
 61. Möbs C, Slotosch C, Löffler H, Jakob T, Hertl M, Pfützner 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.
 62. Jeannin P, Lecoanet S, Delneste Y, Gauchat JF, Bonnefoy JY. IgE versus IgG4 production can be differentially regulated by IL-10. *J Immunol* 1998; **160**:3555-61.
 63. Royer B, Varadaradjalou S, Saas P, Guillosson JJ, Kantelip JP, Arock M. Inhibition of IgE-induced activation of human mast cells by IL-10. *Clin Exp Allergy* 2001; **31**:694-704.
 64. Akdis CA, Joss A, Akdis M, Faith A, Blaser K. A molecular basis for T cell suppression by IL-10: CD28-associated IL-10 receptor inhibits CD28 tyrosine phosphorylation and phosphatidylinositol 3-kinase binding. *FASEB J* 2000; **14**:1666-8.
 65. Wambre E, DeLong JH, James EA, LaFond RE, Robinson D, Kwok WW. Differentiation stage determines pathologic and protective allergen-specific CD4+ T-cell outcomes during specific immunotherapy. *J Allergy Clin Immunol* 2012; **129**:544-51, 551.
 66. Plewako H, Arvidsson M, Oancea I, Hasséus B, Dahlgren U, Rak S. The effect of specific immunotherapy on the expression of costimulatory molecules in late phase reaction of the skin in allergic patients. *Clin Exp Allergy* 2004; **34**:1862-7.
 67. Yanaba K, Bouaziz J-D, Haas KM, Poe JC, Fujimoto M, Tedder TF. A regulatory B cell subset with a unique CD1dhiCD5+ phenotype controls T cell-dependent inflammatory responses. *Immunity* 2008; **28**:639-50.
 68. Haas KM, Watanabe R, Matsushita T *et al*. Protective and pathogenic roles for B cells during systemic autoimmunity in NZB/W F1 mice. *J Immunol* 2010; **184**:4789-800.
 69. Amu S, Saunders SP, Kronenberg M, Mangan NE, Atzberger A, Fallon PG. Regulatory B cells prevent and reverse allergic airway inflammation via FoxP3-positive T regulatory cells in a murine model. *J Allergy Clin Immunol* 2010; **125**:1114-8.
 70. Iwata Y, Matsushita T, Horikawa M *et al*. Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. *Blood* 2011; **117**:530-41.
 71. Noh J, Noh G, Kim HS, Kim A-R, Choi WS. Allergen-specific responses of CD19(+)CD5(+)Foxp3(+) regulatory B cells (Bregs) and CD4(+)Foxp3(+) regulatory T cell (Tregs) in immune tolerance of cow milk allergy of late eczematous reactions. *Cell Immunol* 2012; **274**:109-14.
 72. van de Veen W, Stanic B, Yaman G *et al*. IgG4 production is confined to human IL-10-producing regulatory B cells that suppress antigen-specific immune responses. *J Allergy Clin Immunol* 2013; **131**:1204-12.
 73. 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.
 74. 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.
 75. 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.
 76. 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.
 77. Klimek L, Dormann D, Jarman ER, Cromwell O, Riechelmann H, Reske-Kunz AB. Short-term preseasonal birch pollen allergoid immunotherapy influences symptoms, specific nasal provocation and cytokine levels in nasal secretions, but not peripheral T-cell responses, in patients with allergic rhinitis. *Clin Exp Allergy* 1999; **29**:1326-35.
 78. Hamid QA, Schotman E, Jacobson MR, Walker SM, Durham SR. Increases in IL-12 messenger RNA+ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J Allergy Clin Immunol* 1997; **99**:254-60.
 79. Shamji MH, Ljørring C, Francis JN *et al*. Functional rather than immunoreactive levels of IgG4 correlate closely with clinical response to grass pollen immunotherapy. *Allergy* 2012; **67**:217-26.
 80. Shamji MH, James LK, Durham SR. Serum immunologic markers for monitoring allergen-specific immunotherapy. *Immunol Allergy Clin North Am* 2011; **31**:311-23.
 81. James LK, Shamji MH, Walker SM *et al*. Long-term tolerance after allergen immunotherapy is accompanied by selective persistence of blocking antibodies. *J Allergy Clin Immunol* 2011; **127**:509-16.e1-5.
 82. 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.
 83. Shamji MH, Francis JN, Wurtzen PA, Lund K, Durham SR, Till SJ. Cell-free detection of allergen-IgE cross-linking with immobilized phase CD23: Inhibition by blocking antibody responses after immunotherapy. *J Al-*

- ergy Clin Immunol* 2013;**132**:1003-4.
84. Movérare 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.
 85. Jutel M, Akdis M, Budak F *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**:1205-14.
 86. Cooke RA, Barnard JH, Hebdal S, Stull A. Serologic evidence of immunity with coexisting sensitization in a type of human allergy (hay fever). *J Exp Med* 1935;**62**:733-50.
 87. Lichtenstein LM, Norman PS, Winkenwerder WL. Antibody response following immunotherapy in ragweed hay fever: Allpyral vs. whole ragweed extract. *J Allergy* 1968;**41**:49-57.
 88. Platts-Mills TA, von Maur RK, Ishizaka K, Norman PS, Lichtenstein LM. IgA and IgG anti-ragweed antibodies in nasal secretions. Quantitative measurements of antibodies and correlation with inhibition of histamine release. *J Clin Invest* 1976;**57**:1041-50.
 89. 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.
 90. 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.
 91. Shamji MH, Wilcock LK, Wachholz PA *et al.* The IgE-facilitated allergen binding (FAB) assay: validation of a novel flow-cytometric based method for the detection of inhibitory antibody responses. *J Immunol Methods* 2006;**317**:71-9.
 92. 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.
 93. Varga E-M, Kausar F, Aberer W *et al.* Tolerant beekeepers display venom-specific functional IgG4 antibodies in the absence of specific IgE. *J Allergy Clin Immunol* 2013;**131**:1419-21.
 94. 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**:131-8.e1-7.