

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

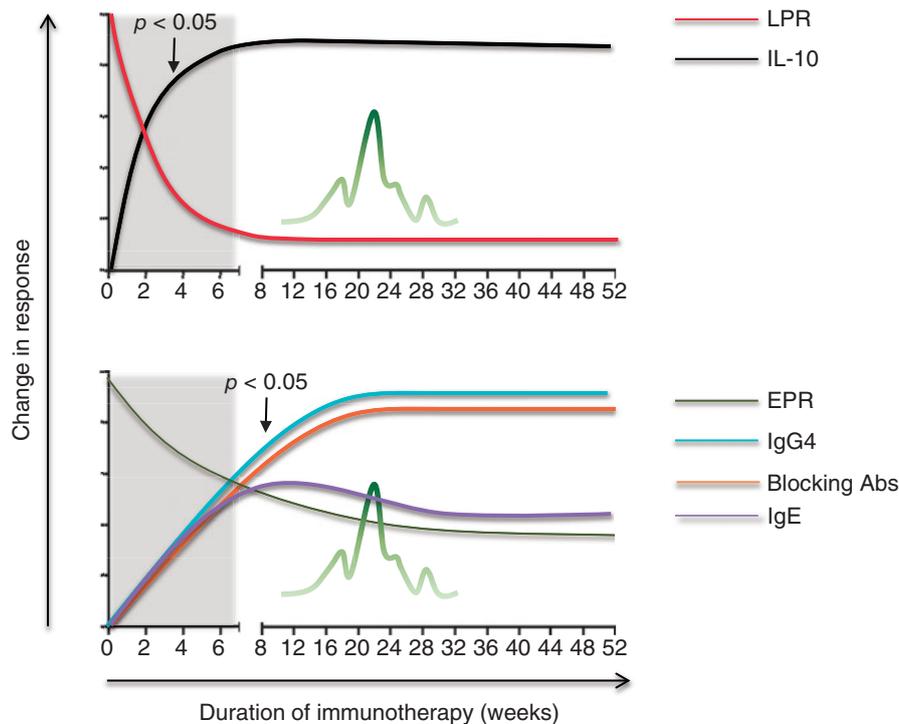


Fig. 1 Time course of response to subcutaneous immunotherapy. Suppression of the late allergic response (LPR) at weeks 2-4 is associated with an increase in IL-10 production. Inhibition of the early allergic responses (EPR) is observed at weeks 8-16 and parallels the induction of immunoreactive IgG4 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

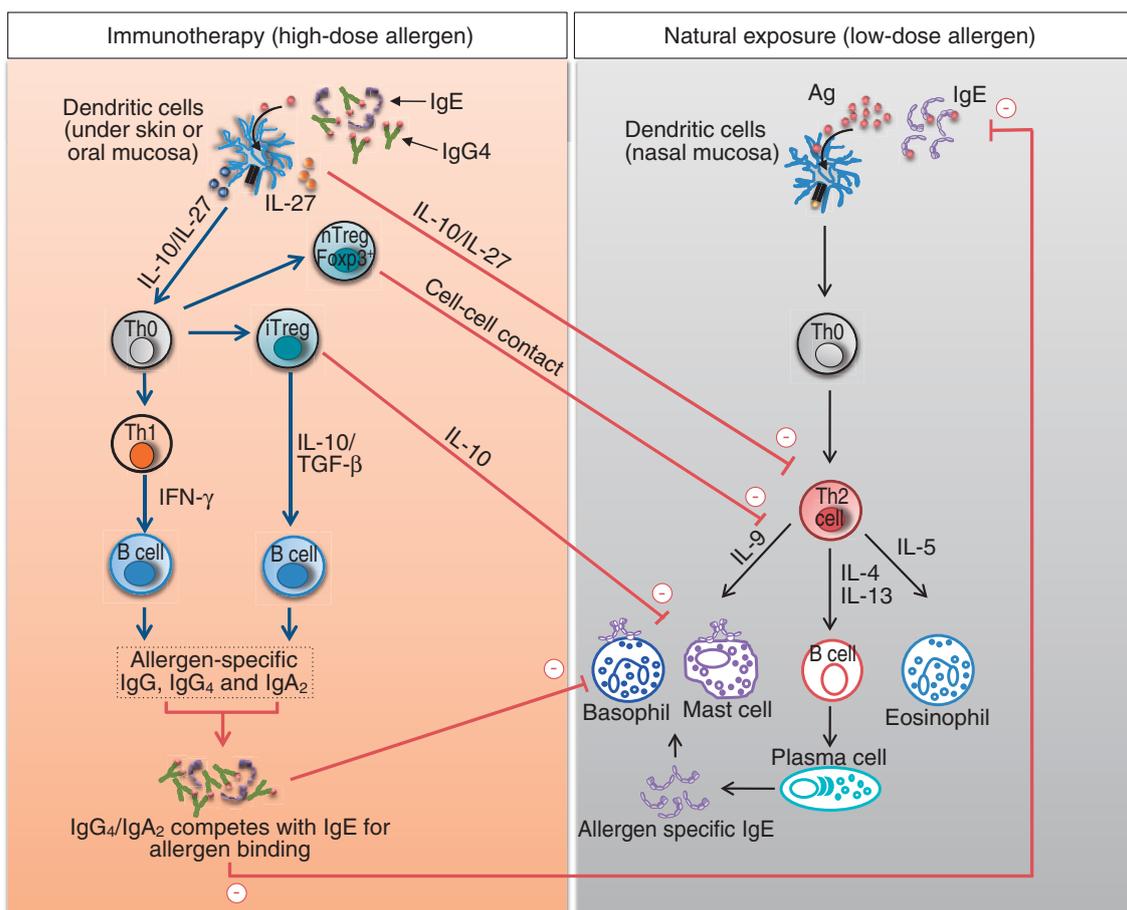


Fig. 2 Mechanisms of immunological and clinical Tolerance in AIT. Low-dose and repeated allergen exposure at mucosal surfaces in atopic individuals drives IgE-facilitated antigen presentation and Th2-driven allergic inflammation. High-dose allergen administered by sublingual or subcutaneous immunotherapy results in immune deviation from a Th2 to a Th1-driven 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). There is also an induction of T regulatory cells [inducible Treg cells (iTreg) and natural Treg cells (nTreg)] and an increase in the regulatory cytokines IL-10, TGF- β and IL-27 following immunotherapy that play an important role in suppressing Th2 responses and contribute towards the induction of allergen-specific IgA₂ and in particular IgG₄ antibodies with inhibitory activity. IgG₄ antibodies are able to compete with IgE for allergen and thereby suppress mast cell and basophil activation and inhibit IgE-facilitated presentation of allergen-IgE complexes to dendritic cells and/or B cells.

allergen-driven Th2 responses during and after AIT.⁴²⁻⁴⁴ Tr1 cells inhibit allergen-driven proliferative T cell responses and Th2 cytokine release.⁴⁵ Whether this suppression is IL-10-dependent is controversial.^{43,45} Natural Tregs were first described by Sakaguchi.^{46,48,49} They are identified by increased expression of the intracellular transcription factor FOXP3 together with a high expression of the cell surface receptor IL-2 receptor (CD25) and low expression of IL-7 receptor (CD127).

In a randomized controlled study of SLIT with a combination of house dust mite and grass pollen extracts, the epigenetic modification of Treg cells was assessed. Methylated CpG sites within the Foxp3 locus of enriched memory CD45RO⁺Treg cells were

enumerated before and 12 months following immunotherapy. DNA methylation was decreased in CD45RO⁺Treg cells at the FOXP3 locus in subjects after 12 months treatment with dual AIT whereas no changes in FOXP3 locus DNA methylation were observed in CD45RO⁺Treg cells in either placebo-treated allergic participants nor healthy normal controls.⁴⁷ These findings support the notion that FoxP3 Tregs are induced following AIT and that this property of T regulatory cells is confined to the memory T cell compartment.

The inhibitory characteristics nTregs *in vitro* and *in vivo* have been described in numerous studies.^{48,49} In addition to the release of soluble inhibitory mediators and cytokines regulation by nTregs is thought to

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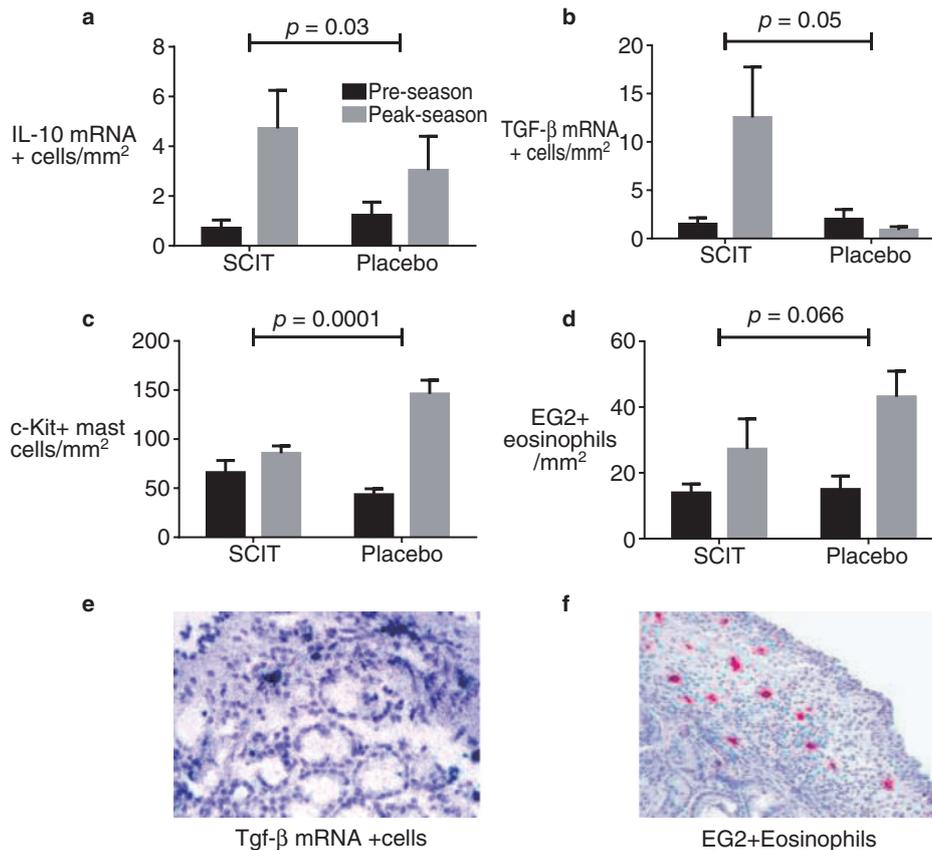


Fig. 3 Effects of 2 years grass pollen subcutaneous immunotherapy on seasonal changes in the nasal mucosa during the pollen season. By *in situ* hybridisation there were significant increases in cells expressing mRNA for the regulatory cytokines IL-10 and TGF-β during the pollen season after immunotherapy 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-β⁴⁹ 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 FcεRI 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.

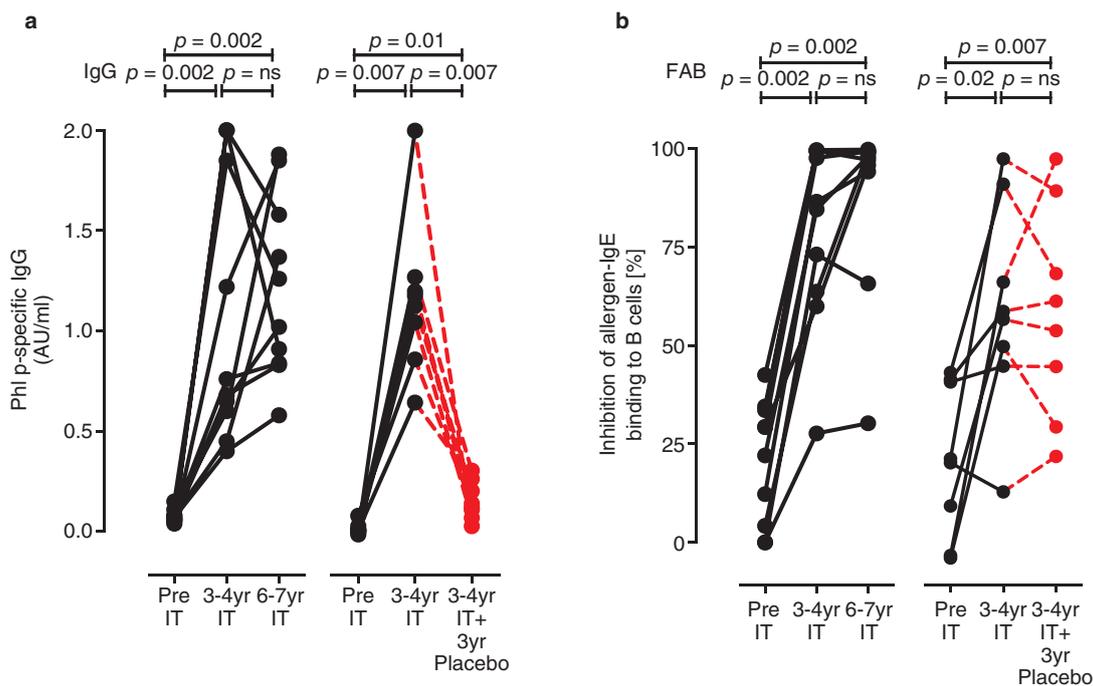


Fig. 4 Persistence of allergen-specific IgG associated inhibitory antibodies after discontinuation of AIT. **(a)** Steady increase in *P. pratense*-specific IgG antibodies in the AIT-treated group (who received 6-7 yr AIT) and a marked decrease in the AIT withdrawn group (who received 3-4 yr AIT followed 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.

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