

# Long-term clinical and immunological effects of allergen immunotherapy

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## Purpose of review

The present review updates current findings on long-term clinical and immunological outcomes after cessation of allergen immunotherapy for allergic respiratory disease.

## Recent findings

Recent studies have shown that allergen immunotherapy has sustained disease-modifying effects that persist for years after discontinuation. This is in contrast to the effects of antiallergic drugs that do not induce tolerance to offending allergens. Long-term effects of immunotherapy include a reduction in nasal symptoms, a decrease in the use of rescue medication and improvement in quality of life. These benefits are accompanied by immunological changes such as the induction of allergen-specific IgG antibodies with inhibitory activity for IgE-facilitated binding of allergen–IgE complexes to B cells. One study reported a reduction in the development of asthma in children with seasonal pollen-induced rhinitis.

## Summary

Allergen immunotherapy induces clinical and immunological tolerance as defined by persistence of clinical benefit and associated long-term immunological parameters after discontinuation of treatment. These findings are largely confined to studies of subcutaneous and sublingual immunotherapy for seasonal pollinosis. Further studies are needed to address potential long-term clinical effects for other seasonal and perennial inhaled allergens in both children and adults, and to identify potential biomarkers of tolerance.

## Keywords

allergen immunotherapy, clinical tolerance, immunological tolerance, long-term effects, sustained efficacy

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## Introduction

The prevalence of allergic diseases such as allergic rhinitis and asthma has increased in the past three decades in both children and adults [1]. In developed countries, more than 20% of the adult population suffers from rhinitis, which represents an independent risk factor for the development of asthma and has a considerable impact on quality of life [2,3]. Options to treat allergic rhinitis/asthma are avoidance of allergen where possible and the use of symptomatic antiallergic drugs that include antihistamines and topical nasal corticosteroid [4,5].

The use of allergen immunotherapy has been extensively reviewed in practice parameters concerning its indication, optimal patient selection for immunotherapy and different treatment protocols concerning both dose and duration of treatment [6–8]. Systematic reviews and meta-analyses have confirmed the efficacy of both subcutaneous [9,10] and sublingual immunotherapy [11,12]

for seasonal and perennial disease, findings that have been confirmed in more recent, ‘definitive’, large, multi-centre trials in adults and children [13–15].

Many valuable lessons have been learned from the field of organ transplantation tolerance and recently it has been demonstrated that B cells are involved in establishing long-term transplantation tolerance [16]. This review will focus on the sustained clinical and immunological outcomes observed after the cessation of allergen immunotherapy.

## Induction of tolerance by allergen immunotherapy

The underlying immunological mechanisms of allergen immunotherapy are pertinent for a better understanding of human antigen-specific tolerance.

Subcutaneous immunotherapy has been shown to be clinically effective in treating allergic rhinitis and/or

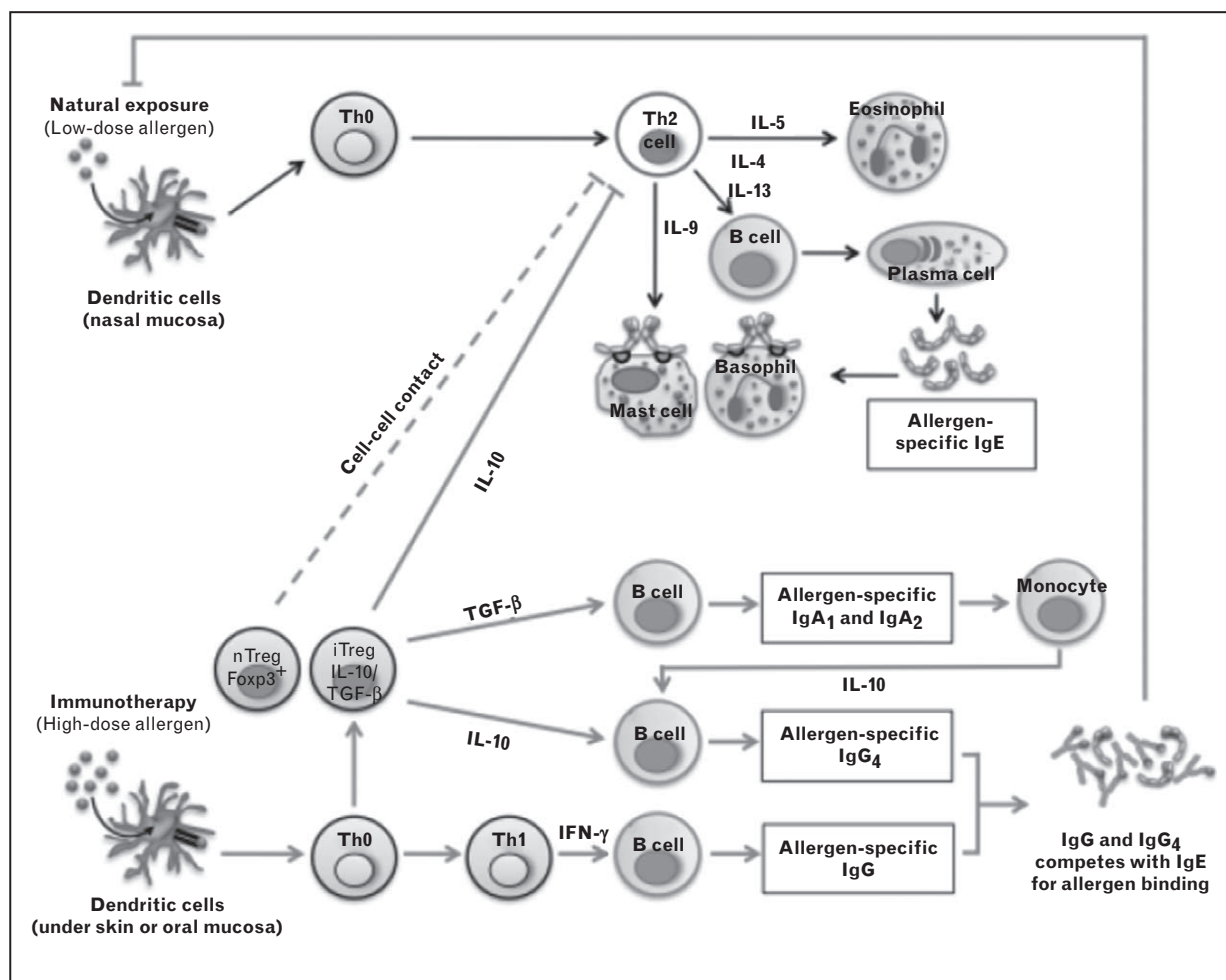
asthma [6,17] with such effects involving T, B and/or T-regulatory cells (Fig. 1) [18]. Subcutaneous immunotherapy in patients with seasonal allergic rhinitis has been shown to blunt seasonal increases in allergen-specific IgE with an increase in both allergen-specific IgG and IgA antibodies [19,20]. Furthermore, immunotherapy results in an increase in serum inhibitory activity for IgE-facilitated binding of allergen–IgE complexes to B cells, an effect mediated by blocking ‘antibodies’ of the IgG class, particularly IgG4 [21]. A number of effector cells are decreased at mucosal sites after immunotherapy, both during natural seasonal allergen exposure and after allergen challenge [22,23]. Recent studies have demonstrated changes in T-lymphocyte responses which might lead to either immune deviation, in favour of Th1 responses with an overproduction of IFN- $\gamma$  [24], and/or an upregulation of T-regulatory cells secreting the inhibitory cytokines IL-10 and/or transforming growth factor beta (TGF- $\beta$ ) that have the potential to suppress allergen-specific Th2 responses

### Key points

- Allergen immunotherapy in grass pollen allergic patients for 3–4 years may result in long-term clinical benefit and persistent immunological effects.
- Regulatory T cells and the induction of B-cell switching in favour of ‘protective’ IgG antibodies with IgE-blocking activity may be one mechanism in the induction of tolerance (persistence of benefit following discontinuation of treatment).
- Further studies are needed to identify other potential surrogate markers to predict the response to immunotherapy and potential for remission/relapse following treatment withdrawal.

[25,26]. The presence of local regulatory T cells was demonstrated by the detection of increased Fox P3<sup>+</sup> T cells in the nasal mucosa after pollen immunotherapy [27].

**Figure 1** A summary of mode of action of allergen immunotherapy



Adapted from [18].

Likewise, sublingual immunotherapy exerts its effects starting on the uptake of allergens by oral mucosal Langerhans cells through high-affinity surface IgE receptors [28,29]. Sublingual immunotherapy reduced antigen-driven T cell proliferation during the early months of treatment. The possibility that IL-10 and/or TGF- $\beta$  could mediate this suppression was elegantly shown by strategies to neutralize these cytokines [30,31]. Scadding *et al.* [32<sup>•</sup>] recently observed immunological effects on local oral mucosal tissues of sublingual immunotherapy treated patients compared to placebo. An increase in sublingual Fox P3<sup>+</sup>-expressing cells was accompanied by an increase in allergen-specific IgG4, IgA and serum inhibitory activity for IgE-facilitated allergen to B cells, changes consistent with those observed following subcutaneous immunotherapy.

Recent studies in other fields have elucidated the mechanisms of antigen-specific tolerance. Studies of immunological tolerance following solid organ transplantation (i.e. stable graft function following withdrawal of immunosuppressive treatment) are an example. Brouard *et al.* [33] used gene-expression arrays to identify series of genes that could distinguish operational tolerance in kidney transplant recipients compared with patients with acute/chronic allograft rejection and with age-matched, healthy control individuals. Although mRNA levels of the TGF- $\beta$  were not upregulated in tolerant patients, approximately 27% of TGF- $\beta$  regulated genes that define naturally acquired tolerance signature were upregulated. Furthermore, two recent studies [34<sup>•</sup>,35<sup>•</sup>] showed an increase in expression of peripheral blood B-cell-related genes [TCL1A and MS4A1 (CD20)] in operationally tolerant patients compared with those using immunosuppressive therapy. On the other hand, studies on peripheral blood of liver transplant recipients were compared between patients successfully tolerized (not under immunosuppressant) with those who had acute rejection and with normal healthy controls. The expansion of T-regulatory (CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> cells), natural killer cells and  $\gamma\delta$  TCR<sup>+</sup> T cells distinguished tolerized from nontolerized recipients, thereby having potential as predictive biomarkers [36,37]. These findings might shed light on mechanisms of tolerance after allergen immunotherapy.

### Clinical and immunological changes during allergen immunotherapy

Clinical endpoints include symptoms and medications scores, disease-specific quality of life questionnaire scores, visual analogue scores and global assessments by patients or physicians before/after treatment. Potential clinical surrogate markers for monitoring allergen-specific immunotherapy include immediate skin prick test responses and the conjunctival/nasal and/or bronchial

response to allergen challenge before/postimmunotherapy and/or compared with healthy controls. Potential immunological biomarkers include serum total and allergen-specific IgE, IgG4 and IgA, changes in T cell proliferation, and allergen-induced Th1, Th2 and T-regulatory cytokines [38,39].

The onset of action of allergen immunotherapy has been studied by the use of an environmental allergen challenge chamber. Patients treated showed significant improvement of rhinitis symptoms compared with placebo as early as 4 weeks after initiation of treatment [40]. Allergen immunotherapy has also been shown to significantly reduce skin, nasal and bronchial-specific allergen responsiveness as early as after 3 months of treatment compared to placebo or conventional pharmacotherapy [25,41,42<sup>•</sup>].

A significant increase of serum-specific IgG4 [25,32<sup>•</sup>,43] and serum allergen-specific IgA after allergen immunotherapy were associated, respectively, with increases in IL-10 and TGF- $\beta$  [19,20,26]. Furthermore, the local demonstration of FOXP3<sup>+</sup>CD25<sup>+</sup> T-cells in the nasal and sublingual mucosa [27,32<sup>•</sup>] after immunotherapy, compared to placebo, further supports a potential role for T-regulatory cells in the induction of allergen-specific tolerance in humans. Collectively, these studies following both subcutaneous and sublingual immunotherapy implicate a possible early role for allergen-specific T-regulatory cells and inhibitory antibodies in the process of early induction of immunological tolerance.

### Long-term clinical effect of allergen immunotherapy after discontinuation

The duration of subcutaneous immunotherapy needed for long-term efficacy after discontinuation of treatment has been generally suggested to be 3–4 years. These studies demonstrated that subcutaneous immunotherapy given for a short time was associated with relapse of symptoms after 1 year of discontinuation compared with the treatment given for more than 3–4 years [44–46]. Subsequently, studies [47,48] of children with allergic rhinitis children who were given 3-year duration of allergen immunotherapy reported a significant improvement in rhinoconjunctivitis symptoms, reduction of conjunctival sensitivity, suppression of new sensitizations and preventive effects on asthma development that persisted for years after stopping treatment.

A recent house dust mite study [49<sup>•</sup>] was designed to assess possible differences in long-term effect of either 3 or 5 years' duration of subcutaneous immunotherapy as recommended. After 3 years' treatment with house dust mite with the first year having a double-blind, placebo controlled phase, one group was then followed for 2 years

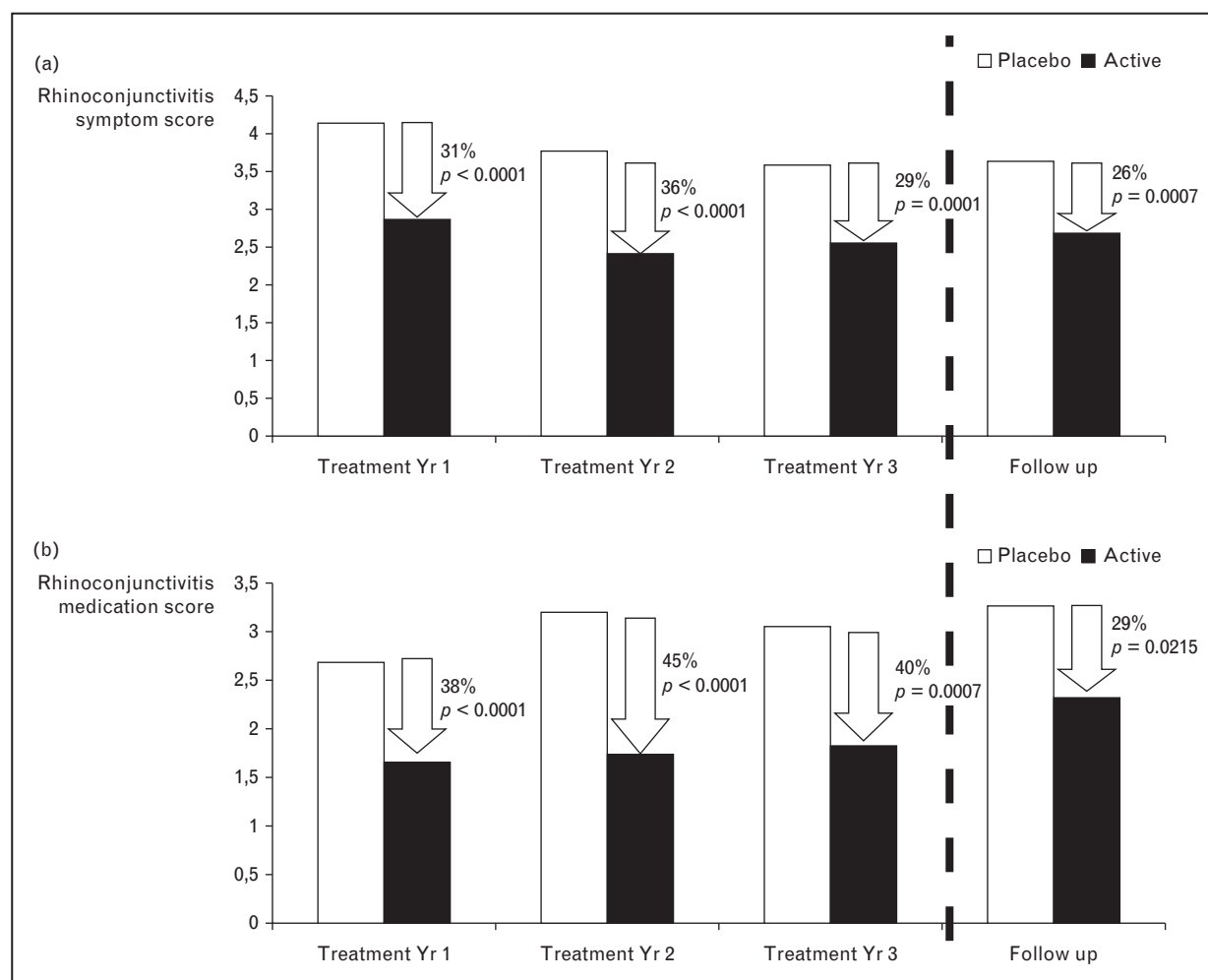
without treatment, whereas the other group continued with maintenance to complete 5 years of treatment. Clinical efficacy was evaluated in 240 patients at the end of follow-up. After 3 and 5 years of treatment, both groups had significant improvement of symptoms compared with baseline, with more than 70% reduction in rhinitis symptoms in the 5-years group compared with 50% reduction in the 3-years treatment group, with comparable reduction in visual analogue scale and increase in quality of life. There were no statistical differences between the 3-year or 5-year immunotherapy-treated groups in terms of global rhinitis and asthma symptoms. These observations suggest that 3 years of treatment is sufficient to induce long-term effect of at least 2 years.

Sublingual immunotherapy has emerged to be an effective alternative to the subcutaneous route and is widely

used in Europe [50]. Several single-centre, small size studies in children and adults compared sublingual and subcutaneous immunotherapy [41,51] and showed similar efficacy between these two routes. However, these small studies were insufficient to assess tolerance after sublingual treatment.

The first, large-scale, multicentre, double-blind, placebo-controlled, randomized trial of sustained efficacy of sublingual immunotherapy after 3 years of treatment recruited over 600 patients. Participants were followed double-blind for 2 years after discontinuation. The results following 1 year of blinded withdrawal are now published [52<sup>\*</sup>]. Compared with placebo, active treatment at 1 year after cessation of treatment had a significant reduction of 26% of mean average daily rhinoconjunctivitis symptom and 29% of medication scores (Fig. 2) [52<sup>\*</sup>]. These outcomes were similar to

**Figure 2 Long-term clinical efficacy of allergen immunotherapy**

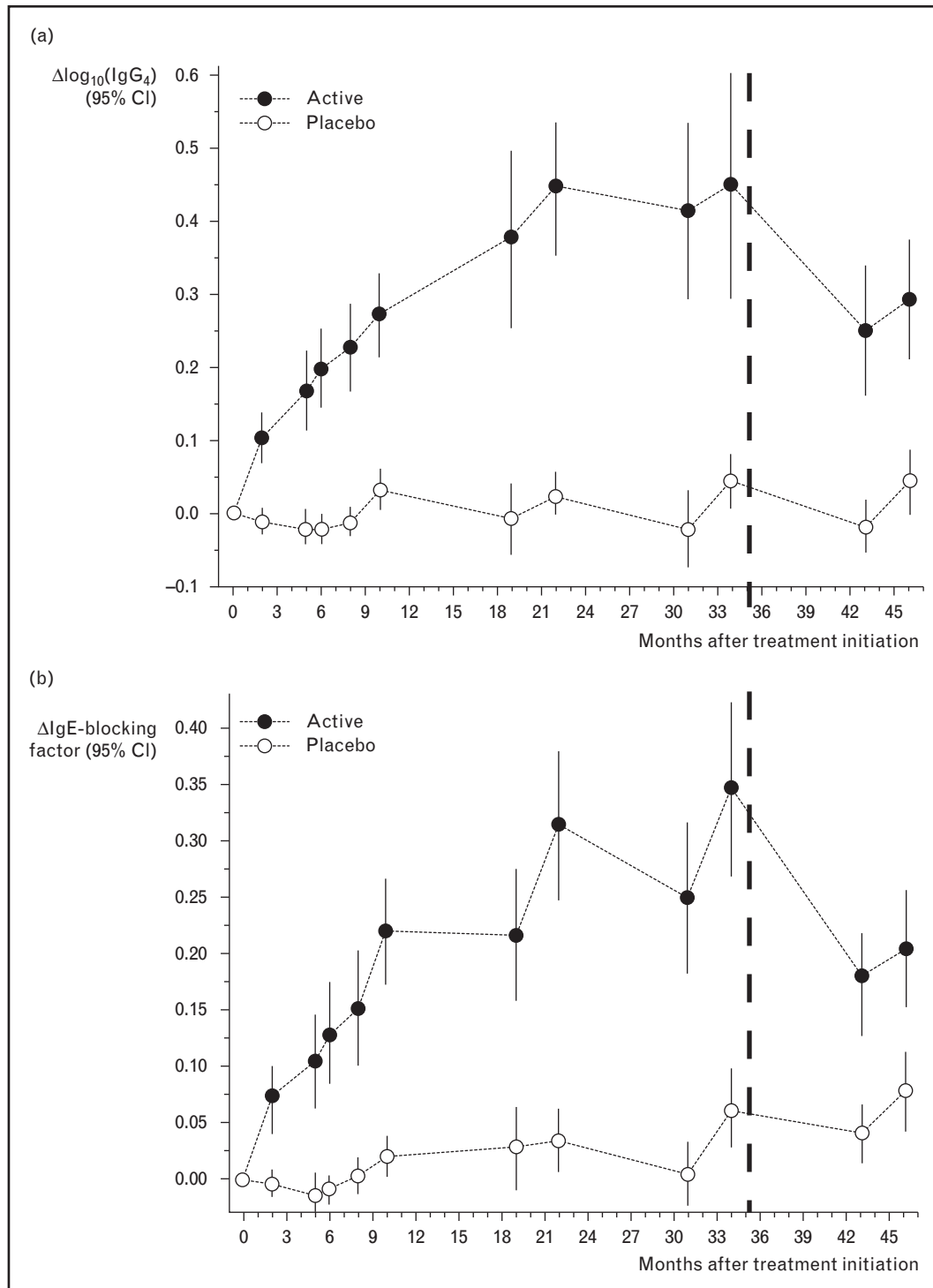


After the 3 years of allergen immunotherapy, compared with placebo, active treatment at 1 year after cessation of treatment had a significant reduction of both (a) rhinoconjunctivitis symptom and (b) medication usage. The dotted line symbolizes the end of 3 years of treatment. Adapted from [52<sup>\*</sup>].

those observed during the 3-year treatment period. Moreover, active treatment showed a significant improvement in symptom and medication-free days with a better quality of life during the pollen seasons after 1 year of

discontinuation [52<sup>\*</sup>]. These clinical effects in the actively treated group were sustained with a 25% reduction in adjusted mean symptom scores and 27% in combined score 2 years after the treatment completion [53].

**Figure 3 Persistence immunological changes after cessation of allergen immunotherapy**



Differences in change of both (a) specific IgG<sub>4</sub> and (b) IgE blocking factor from baseline were persistently and significantly different 1 year after sublingual immunotherapy. Adapted from [52<sup>\*</sup>].

Ott *et al.* [54] reported a trend for long-term efficacy of sublingual immunotherapy compared with placebo in a study that included a baseline year and used a co-seasonal ultrarush protocol for 3 consecutive years and a 1-year follow-up period. These observations were partly confounded by the groups being mismatched for seasonal symptoms during a baseline year by chance.

There are very few studies that evaluated clinical outcomes of more than 5 years after stopping sublingual immunotherapy mostly in open, nonrandomized controlled studies. A recent, open, partially randomized study [55<sup>•</sup>] which prospectively evaluated the long-term effect of sublingual treatment in an adult population according to treatment duration (3, 4 or 5 years), compared to pharmacotherapy alone. With a minimum duration of 3 years, clinical efficacy could be demonstrated up to 5 years after immunotherapy treatment cessation compared to controls, whereas following both 4 and 5 years of treatment sustained efficacy could be seen 7 years after stopping treatment. However, the small numbers studied and only partially randomized design are limitations to the interpretation of results.

### Long-term immunological effects after discontinuing allergen immunotherapy

Few studies have reported long-term immunologic outcomes after discontinuation of allergen immunotherapy. The effects of IL-10 in induction of peripheral tolerance includes class switch of B cells towards IgG4, which have inhibitory effects by blocking activity on IgE-facilitated binding to B cells and the inhibition of basophil histamine release [25,39,56]. Previously, it has been demonstrated that sustained clinical efficacy after grass pollen subcutaneous immunotherapy is associated with continuing suppression of allergen-induced, late-phase, skin responses together with the inhibition of local expression of mRNA for Th2 cytokines [44]. In a recent controlled study [57<sup>•</sup>] of subcutaneous immunotherapy, long-term clinical tolerance after discontinuation of treatment was associated with the persistence of IgG-associated serum inhibitory activity against binding of IgE–allergen complexes to B cells, a marker of IgE-facilitated antigen presentation. This effect persisted even after 2 years of treatment discontinuation, which occurred in the face of a decline in allergen-specific IgG1 and IgG4 compared to during the treatment.

In a recent study of sublingual grass pollen immunotherapy [52<sup>•</sup>,53], a progressive parallel increase in both specific IgG4 and IgE blocking activity was observed in the immunotherapy-treated group that was sustained throughout an observation period of 2 years

after discontinuation (Fig. 3) [52<sup>•</sup>], suggesting a possible mechanism for the long-term persistence of allergen-specific tolerance to grass pollen. This was not observed in the study by Ott *et al.* [54], which reported that the clinical improvement 1 year after stopping treatment was not correlated with specific IgG4 levels. However, in this study [54], assays of the functional activity of IgG were not included.

Studies of venom immunotherapy have demonstrated that after cessation of 5 years treatment, in spite of repeated sting challenges every after 2–4 years, venom skin prick and specific IgE declined in a linear fashion during 1–5 years of observation [58].

Recently, in order to assess possible long-term tolerance 2 years after discontinuation of venom immunotherapy in children, the time course of serum inhibitory activity for IgE-facilitated allergen binding and venom-specific IgG4 was studied. Persistent decreases in venom-specific IgE were observed, whereas sustained increases in serum inhibitory activity and specific IgG4 were not. Although further studies are needed, these findings raise the possibility that a progressive decrease in venom-specific IgE rather than a sustained increase in IgG-blocking activity may be responsible for long-term tolerance following venom immunotherapy [59].

### Conclusion

Allergen immunotherapy given for 3–4 years for grass pollen allergy, via both subcutaneous and sublingual routes, appears adequate to induce long-term tolerance after discontinuation as reflected by improved symptoms, a reduction in progression to asthma, and prevention of new sensitizations. One possible explanation for this long-term effect may be the persistence of IgG-associated serum inhibitory activity against specific IgE. Further research is required to identify other surrogate markers that might correlate with the clinical response to immunotherapy and predict persistent improvement (tolerance) or relapse after discontinuation of immunotherapy.

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#### Conflicts of interest

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A.O.E. and M.H.S. have no conflicts of interest to declare.

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