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Applications and mechanisms of immunotherapy in allergic rhinitis and asthma

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Abstract: Clinical and immunologic tolerance are hallmarks of successful allergen immunotherapy (AIT). Clinical benefits such as reduced symptoms, pharmacotherapy intake and improvement of quality of life persist following cessation of treatment. Successful AIT is associated with suppression of allergic inflammatory cells such as mast cells, eosinophils and basophils in target organs. Furthermore, AIT down-regulates type 2 innate lymphoid cells and allergen-specific type 2 T-helper (Th2) cells. The immunologic tolerant state following AIT is associated with the induction of distinct phenotypes of regulatory T-cells (T-regs) including interleukin (IL)-10-, IL-35- and transforming growth factor (TGF)-β- producing T-regs and FoxP3$^+$ T-regs. B-cell responses, including the induction of IL-10$^+$ regulatory B-cells (B-regs) and the production of IgG4-associated blocking antibodies are also induced following successful AIT. These events are associated with the suppression of antigen-specific Th2 responses and delayed immune deviation in favour of Th1 type responses. Insight into the mechanisms of AIT has allowed identification of novel biomarkers with potential to predict the clinical response to AIT and also novel therapeutic strategies for more effective and safer AIT.

Keywords: allergen immunotherapy, allergic rhinitis, allergy, asthma, biomarkers, blocking antibodies, dendritic cells, IgA, IgG4, regulatory B-cells, regulatory T-cells, tolerance

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Introduction

Allergic rhinitis (AR) and asthma are common diseases with prevalence estimates of up to 30% for AR and 10–20% for asthma [Bauchau and Durham, 2004; Eder *et al.* 2006; Bernstein, 2010; Sullivan *et al.* 2011; Martinez and Vercelli, 2013]. Most patients with asthma have AR while up to 40% of patients with AR also have asthma comorbidity [Cruz *et al.* 2007; Compalati *et al.* 2010; Braunstahl, 2011]. Moreover, AR is an independent risk factor in the development of asthma [Guerra *et al.* 2002]. In western countries, allergic disorders such as AR and asthma have a considerable impact on quality of life [Bauchau and Durham, 2004; Eder *et al.* 2006; Sullivan *et al.* 2011]. They affect school performance in children and work productivity in adults [Simons, 1996; Cockburn *et al.* 1999; Sullivan *et al.* 2011]. Disease burden is mainly caused by responses on exposure to common aeroallergens derived from grass, birch, pet or house dust mite [Bousquet *et al.* 2001; Bauchau and Durham, 2004].

Allergen avoidance and pharmacotherapy such as antihistamines and local corticosteroids are the first line treatment management for controlling symptoms in AR [Bousquet *et al.* 2012; Braido *et al.* 2014; Jutel, 2014; Scadding, 2015; Wheatley and Togias, 2015]. For asthma control, long acting bronchodilators (LABAs) and inhaled corticosteroids (ICSs) are recommended by current guidelines [Reddel *et al.* 2015a, 2015b]. Although therapies for AR with or without asthma have proven effective in controlling symptoms and reducing inflammation, a small proportion of patients do not respond to pharmacotherapy and in these patients, allergen-specific immunotherapy may be of importance.

Allergen immunotherapy (AIT), first reported by Leonard Noon in the early 20th century, is a highly effective treatment in individuals with immunoglobulin (Ig)E-mediated diseases [Bousquet *et al.* 1998; Durham *et al.* 1999; Calderon *et al.* 2007; Shamji *et al.* 2012]. AIT is associated with the reduction of symptoms [Calderon *et al.* 2007, 2011; Compalati *et al.* 2014], a reduction in the use of rescue medications, improvement of quality of life [Malling and Bousquet, 2008; Burks *et al.* 2013; Canonica *et al.* 2014; Jutel *et al.* 2015] and induces clinical and immunological allergen-specific immune tolerance [Bousquet *et al.* 1998; Durham *et al.* 1999; Bousquet *et al.* 2001; Calderon *et al.* 2007; Bousquet *et al.* 2008; Shamji *et al.* 2012]. AIT can be administered either by a subcutaneous (SCIT) or sublingual (SLIT) route. Although adequate head-to-head studies comparing SLIT and SCIT are limited, there is a tendency to think that SCIT might be superior to SLIT [Di Bona *et al.* 2012; Chelladurai *et al.* 2013; Dretzke *et al.* 2013]. SLIT is considered to have a safer profile compared with SCIT, with a lower number of anaphylactic reactions. However, local side effects have been reported following SLIT [Fujimura and Okamoto, 2010]. AIT is most often applied in mono-allergy but can also be used in multi-allergic patients.

According to the AR and its Impact on Asthma (ARIA) guidelines [Brozek *et al.* 2010; Bousquet *et al.* 2012], AIT is indicated in moderate-to-severe intermittent AR if patients are unresponsive to pharmacotherapy. However, ARIA guidelines only give a conditional recommendation for AIT (both SLIT and SCIT) in allergic asthma due to moderate and low quality evidence [Brozek *et al.* 2010; Bousquet *et al.* 2012], while Global Initiative for Asthma (GINA) guidelines indicate that AIT should be considered after pharmacological treatment and allergen avoidance [Reddel *et al.* 2015a]. Current guidelines state that in clinical practice, uncontrolled asthma is a contraindication for both SCIT and SLIT [Di Bona *et al.* 2012; Pitsios *et al.* 2015].

Effects of allergen immunotherapy on early and late phase responses

Both the early and the late phase allergic responses are suppressed after AIT, starting with the late phase response already 2 weeks after the start of treatment [Francis *et al.* 2008]. Suppression of the IgE-mediated early cutaneous responses is less pronounced and occurs 8–12 weeks after the first dose [Durham *et al.* 1999; Francis *et al.* 2008]. Interestingly, mast cell and basophil degranulation is decreased soon after the first injection of AIT [Iliopoulos *et al.* 1991; Akdis and Akdis, 2015]. In other studies, skin biopsies following AIT have shown reduced numbers of eosinophilic, neutrophilic and basophilic infiltration after AIT [Varney *et al.* 1993; Nish *et al.* 1994]. The suppression of late phase responses have been reported in skin, nose and in the lungs [Warner *et al.* 1978; Pienkowski *et al.* 1985; Iliopoulos *et al.* 1991]. Reduction in nasal responses have also been reported for grass pollen, and cat allergens [Nanda *et al.* 2004; Puggioni *et al.* 2005]. Allergic asthmatics demonstrated a reduction in late responses after AIT in mite and birch studies [Warner *et al.* 1978; Arvidsson *et al.* 2004].

Mechanisms of allergen immunotherapy

A variety of immune cells, such as T-helper lymphocytes type 2 (Th2), eosinophils, mast cells and basophils [Arvidsson *et al.* 2004; Novak *et al.* 2011; Shamji *et al.* 2011], are responsible for the inflammation observed in allergic diseases. Th2 cells play an essential role by producing cytokines such as interleukin (IL)-4, IL-5 and IL-13 that subsequently are responsible for the induction of effector cells. Furthermore, Th2 cells drive B-cells to produce allergen-specific IgE (sIgE). Cellular and humoral responses associated with AIT are presented in Figure 1.

Cellular changes

In allergic individuals, cytokines and mediators such as TSLP, IL-25, IL-33 derived from epithelial cells induce dendritic cells (DCs) to polarize naïve T-cell responses towards a Th2 response [Bellinghausen *et al.* 2001; Hurst *et al.* 2002; Soumelis *et al.* 2002; Ito *et al.* 2005; Schmitz *et al.* 2005]. AIT results in an induction of tolerant DCs that skew naïve T-cells towards IL-10-producing regulatory T-cells (Tregs) subsequently leading to a Th1 response [Shamji *et al.* 2011; Akdis and Akdis, 2015]. Several studies have reported the increase of Th1 cytokines and chemokines such as eotaxin, or tumour necrosis factor (TNF)α, paralleled by an upregulation of Th1 [interferon (IFN)-γ, IL-12] and regulatory cytokines [IL-10, transforming growth factor (TGF)β] [Faith *et al.* 2003; Jutel *et al.* 2003] although others report no changes [Wachholz *et al.* 2002; Francis *et al.* 2003]. It is still believed that these changes in serum cytokines as well as chemokines are immunological

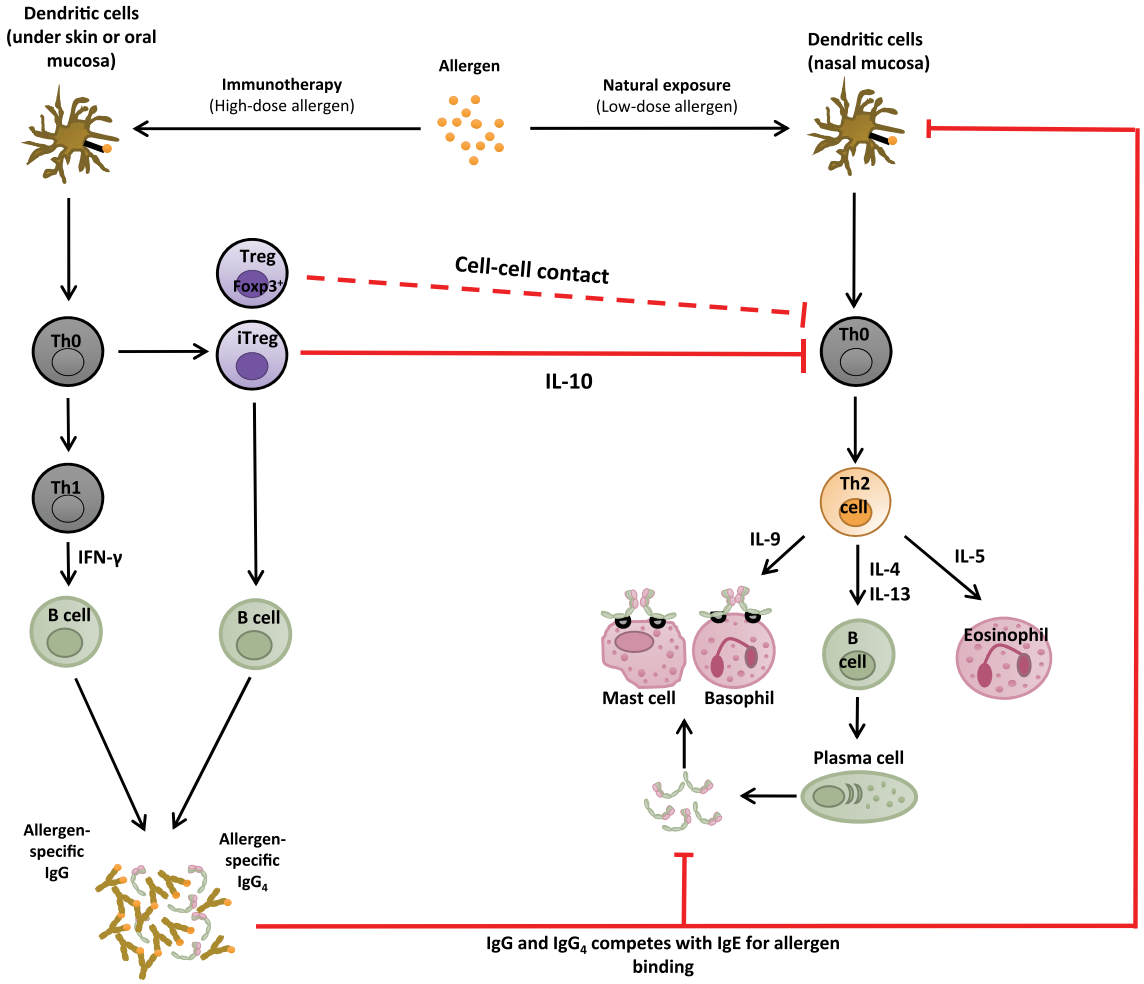

Figure 1. Mechanisms of immunological and clinical tolerance in AIT. Natural (low-dose) allergen exposure at mucosal surfaces results in a Th2-driven allergic inflammation with mast-cells, basophils and eosinophilic activity. High-dose allergen administered by SLIT or SCIT immunotherapy results in immune deviation from a Th2 to a Th1-driven response. This is accompanied by an increase in the ratio of Th1 cytokines such as IFN-γ and an induction of Treg cells and inducible Treg cells (iTregs) and an increase in the regulatory cytokines such as IL-10. Furthermore, AIT leads to allergen-specific IgG, in particular IgG4 antibodies with inhibitory activity.
AIT, allergen immunotherapy; IFN, interferon; Ig, immunoglobulin; IL, interleukin; iTreg, inducible Treg; Th, T-helper lymphocyte; Treg, regulatory T-cells; SCIT, subcutaneous AIT; SLIT, sublingual AIT

paraphenomena of AIT, often not directly correlated with clinical outcome. Also local cytokines indicate a skew towards a more Th1 response; however only a very limited number of studies are available to support these findings [Kirmaz *et al.* 2011; Scadding *et al.* 2015]. Recent data also demonstrate that AIT inhibits the seasonal increase of type 2 innate lymphoid cells [Lao-Araya *et al.* 2014].

A key mechanism in tolerance induction following AIT is the upregulation of peripheral and local allergen-specific Tregs [Bohle *et al.* 2007; Radulovic *et al.* 2008; O'Hehir *et al.* 2009; Rolland *et al.* 2010; Scadding *et al.* 2010; Akdis and Akdis, 2014]. Treg cells can be grouped into two subsets, Foxp3+ Tregs and inducible Tregs (iTregs) that produce regulatory cytokines such as IL-10, TGF-β and IL-35 [Bohle *et al.* 2007; Shamji *et al.* 2012, 2013]. Several studies have suggested that there is an overlap between these subsets of Treg cells in immunomodulating properties [Akdis and Akdis, 2015]. The early induction of Tregs during AIT has been associated with delayed immune deviation from Th2-type response to Th1-type response, mainly because of increased IL-10 production. Also, the association of increased numbers of Tregs in the nasal mucosa

after immunotherapy with positive clinical outcome, as well as the suppression of seasonal allergic inflammation, support a central role for Treg cells in the induction of allergen-specific tolerance [Radulovic *et al.* 2008; Scadding *et al.* 2010]. Recently, epigenetic changes of memory Treg cells during dual house dust mite (HDM) and grass pollen SLIT indicated that methylation of the FOXP3 locus might be involved in the mechanism of allergy tolerance after AIT [Mitra *et al.* 2012].

During AIT, B-cells show a shift from producing the allergenic IgE to the more 'protective' IgG4. Although symptoms decrease from the start of AIT, a transient increase in sIgE is observed in serum. Eventually there is a gradual decrease of sIgE in serum over years of treatment [Van Der Neut Kolfschoten *et al.* 2007]. Specific IgG4 shows a relatively early and rapid increase and continues to increase during AIT. After discontinuation of treatment, sIgG4 decreases again; however it still remains elevated compared with baseline levels [Shamji *et al.* 2011, 2012].

Apart from antibody production, B-cells also contribute to immune responses through antigen presentation to T-cells and secretion of cytokines. For the production of antibodies B-cells differentiate to plasma cells which can reside for many years in in the bone marrow; even independent of exposure to antigens they can produce antibodies. The upregulated IL-10 suppresses antigen presentation by antigen presenting cells as well Th2 production of IL-4 which leads to a lower IgE production by plasma cells. At the B-cell level, IL-10 enhances the survival, proliferation, differentiation and isotype switching of human B-cells resulting in IgA and IgG4 production.

DCs are specialized antigen-presenting cells and can initiate and sustain allergic inflammation, or support tolerance induction. A recent AIT study demonstrated a significant decrease in the number of DCs and an upregulation of regulatory DCs (DCreg) after SLIT in grass pollen-allergic patients [Zimmer *et al.* 2012]. Changes in the DCreg/DC2 balance occur after 4 months of SLIT. Changes in DCs were only identified in those patients with a significant decrease in rhinoconjunctivitis symptoms [Zimmer *et al.* 2012]. After 1 year of SLIT, low production of IL-12 and an increased IL-10 secretion as well as a decreased capacity to mature was observed in HDM-allergic children [Angelini *et al.* 2011].

Basophils consist of 1% of leucocytes in peripheral blood and contain cytoplasmic secretory granules that have an important role in systemic allergic responses [Ishizaka *et al.* 1972; Falcone *et al.* 2011]. Allergen cross-linking of sIgE on basophils initiates degranulation and subsequent release of histamine, leukotrienes, and other mediators of the allergic inflammatory response [Schroeder *et al.* 1995; Macglashan, 2010]. It has been shown that after AIT, basophil activation is inhibited by preventing allergen IgE interaction or the interaction with FcγRIIb by competitive allergen-specific IgG antibodies, including IgG4 [Van Neerven *et al.* 1999; Kepley *et al.* 2000; Wachholz *et al.* 2003].

Recently, a reverse staining technique for basophil activation was used in which phycoerythrin-conjugated diamine oxidase (DAO) binding histamine within the cell was reduced after degranulation of basophils [Ebo *et al.* 2012].

The results obtained with basophil activation during AIT in placebo-controlled studies are conflicting. Some authors describe reduction in basophil activation following AIT possibly due to serological factors [Ceuppens *et al.* 2009; Aasbjerg *et al.* 2014; Ozdemira *et al.* 2014; Schmid *et al.* 2014]. Van Overtvelt and Gomez failed to demonstrate the effects of AIT on basophil activation following AIT [Van Overtvelt *et al.* 2011; Gomez *et al.* 2015].

The suppression of basophil activation after discontinuation of AIT has been demonstrated 12–24 months following discontinuation of treatment [Nopp *et al.* 2009; Lalek *et al.* 2010; Gokmen *et al.* 2012; Shamji *et al.* 2015; Zidarn *et al.* 2015].

Humoral changes

The gold standard for allergy diagnosis and starting AIT is a serum-elevated sIgE in the context of symptoms in exposure to the relevant allergen [Cox *et al.* 2011; Burks *et al.* 2013; Calderon *et al.* 2013]. Many pollen AIT studies demonstrated transient increases of sIgE levels followed by blunting of the seasonal increases. So far, no functional relevance or severe allergic reactions have been associated with this transient increase. In AIT studies with a long duration, the levels of sIgE levels were shown to decrease over time [Nouri-Aria *et al.* 2004; Pilette *et al.* 2007]. For total IgE response during AIT the results are

contradictory. Many studies have reported no change while others have reported an increase or decrease in the levels of IgE.

In the mid-1930s, Cooke reported on the induction of serum inhibitory antibody activity following AIT which later proved to be serum inhibitory activity for IgE [Cooke *et al.* 1935]. It is assumed that mainly antibodies in the IgA and IgG fraction of the serum cause this effect [Lichtenstein *et al.* 1968; Platts-Mills *et al.* 1976]. As a result of AIT, increases in the range of 10–100 fold in the concentrations of IgG1 and particularly of IgG4 are observed [Jutel *et al.* 2005; Reisinger *et al.* 2005]. Some studies were even able to demonstrate, a correlation between allergen sIgG4 and clinical outcomes [Gehlhar *et al.* 1999; Moverare *et al.* 2002; Nelson *et al.* 2011; Gomez *et al.* 2015]. After the initial induction of sIgG4 the levels decrease after discontinuation of treatment, although are still elevated compared with baseline levels. It is thought that IgG4 competes with allergen-binding to the IgE on the Fcε receptors of mast cells and basophils, and thus acts as a blocking antibody that prevents the activation and degranulation of effector cells [Van Neerven *et al.* 1999; Wurtzen *et al.* 2008]. In addition, some other features of IgG4 suggest that it could have an anti-inflammatory role. IgG4 antibodies are dynamic molecules that exchange Fab (facilitating antigen binding) arms by swapping heavy-light chain pairs between IgG4 molecules with different specificities and immune complex formation; whereas they retain their inhibitory activity by competing with IgE for allergens, thereby inhibiting IgE-allergen complex formation [Rispens *et al.* 2011]. This process results in the production of bi-specific antibodies with a substantially decreased capacity for cross-linking [Aalberse and Schuurman, 2002]. In addition, serum 'blocking' IgG4 antibodies have the capacity to suppress both allergen-triggered basophil histamine release and the binding of IgE-allergen complexes to B-cells.

Also, in nasal secretions, IgG levels in allergic individuals are also elevated [Platts-Mills *et al.* 1976]. More recently, sIgG4 antibodies with inhibitory activity for IgE-facilitated antigen binding (IgE-FAB) were measured in the nasal fluid of SLIT patients [Saleem *et al.* 2013; Shamji *et al.* 2013]. It was demonstrated that sIgG4 levels were increased in AIT and that the inhibitory effect was significantly increased compared with untreated patients. An interesting observation was that the magnitude of the suppression was higher in the local antibodies compared with peripheral antibodies [Saleem *et al.* 2013; Shamji *et al.* 2013]. Furthermore, the ratio of IgE/sIgG4 was demonstrated to decrease in several SLIT studies and was correlated with a decrease in late-phase skin reaction [Troise *et al.* 1995; La Rosa *et al.* 1999; Lima *et al.* 2002; Bahceciler *et al.* 2005]; however, this was not a consistent finding [Rolinck-Werninghaus *et al.* 2005]. One study with genetically modified allergens showed IgG1 and IgG4 in mucosal fluids after AIT. The increase of IgG4 levels was significantly associated with a reduction in nasal sensitivity [Reisinger *et al.* 2005].

In allergic individuals, allergen-IgE complexes bind to low-affinity IgE receptor FcεRII (CD23) on the surface of B-cells (IgE-FAB); subsequently activated B-cells would be able to present the allergen to specific T-cell clones. It has been demonstrated in several studies that serum obtained from subjects receiving birch pollen immunotherapy inhibits IgE-facilitated presentation of allergen by B-cells to an allergen-specific T-cell clone [Van Neerven *et al.* 1999; Wurtzen *et al.* 2008]. Furthermore, serum obtained from patients that received grass pollen immunotherapy could inhibit IgE-facilitated allergen presentation to a grass-specific T-cell clone [Wachholz *et al.* 2003]. Specific IgG4 appears to play a key role in this mechanism [Nouri-Aria *et al.* 2004; James *et al.* 2011]. IgE-Fab has been shown to decrease after AIT and is modestly correlated with a clinical response to grass and birch AIT up to 2 years after discontinuation of AIT [Van Neerven *et al.* 1999; Wachholz *et al.* 2003; Durham *et al.* 2012].

Follow up on immunotherapy

Clinical follow up

The efficacy of AIT is evaluated by clinical symptoms and rescue medications scores at the time of natural allergen exposure; however there is no validated and generally accepted method for combining clinical parameters with medication scores. An overview of available methods was presented by Pfaar and colleagues in an European Academy of Allergy and Clinical Immunology (EAACI) task force position paper in 2014 [Pfaar *et al.* 2014]. All advantages and unmet needs of the different methods are listed.

The task force highlights combined symptom and medication scores (CSMS) as primary endpoints for AIT in allergic rhinoconjunctivitis. For assessing the disease from the patients' perspective, the Visual Analogue Score (VAS) is considered to be a useful, easy and (partially) validated measure, to be included in clinical trials on AIT aimed to provide a quantitative evaluation of disease severity [Pfaar *et al.* 2014; Hellings *et al.* 2015].

Biomarkers

Biomarkers are quantitative measurements that predict clinical and immunological effects of AIT in the target organ. Markers can be cellular (e.g. Tregs), humoral (sIgG4, IgE/IgG4), molecular (interleukins) or functional (IgE-FAB and blocking factor). Biomarkers could assist in patient selection, identification of responders, target intervention of those who will benefit and to exclude those who are less likely to respond to AIT. Furthermore, they could be of assistance in clinical trials for the development of treatment modalities. Although an overview and recommendations for the standardization of clinical outcomes used in AIT is available [Pfaar *et al.* 2014], to date there is no consensus on candidate biomarkers that are predictive of the clinical response to AIT [Food and Drug Administration, 2008]. Table 1 presents an overview of studies with a clinical improvement after AIT presenting data on some biomarkers. At present, raised serum sIgE in the context of a clear history of symptoms on exposure to the relevant allergen is the only generally available biomarker for selection of patients for immunotherapy.

The recent availability of allergen micro-arrays has refined the process to assist in the identification of patients with irrelevant cross-reacting IgE-antibodies to pan allergens. An example is selecting patients for grass immunotherapy on the basis of having IgE-antibodies to Phleum p 1 and/or Phleum p 5, whereas a positive skin test with Phleum extract in the context of a positive Phleum p 12 is likely to represent irrelevant cross-reactivity with the birch profilin Bet v 2.

As mentioned above, many cellular and humoral changes are observed during and after AIT. For example with the increase in sIgG4, some studies were even able to demonstrate a correlation between allergen sIgG4 and clinical outcomes [Gehlhar *et al.* 1999; Moverare *et al.* 2002; Nelson *et al.* 2011; Gomez *et al.* 2015]. Although after the initial induction of sIgG4 the levels decrease after discontinuation of treatment, they are still elevated compared with baseline levels.

A promising assay for the follow up of AIT is IgE-FAB, an *in vitro* biofunctional cellular assay that proved to be highly reproducible. It has a within-assay and between-assay reproducibility of 4% and 12% respectively, and can be utilized to detect IgG-associated serum inhibitory activity during as well as after discontinuation of AIT. The assay is highly complex and therefore limited to specialized laboratories. More recently, an alternative and less complex method, an Enzyme-Linked Immunosorbent Facilitated Antigen Binding (ELIFAB) assay, has become available [Shamji *et al.* 2013].

Some studies show a modest correlation with clinical response to grass or birch and IgE-FAB [Van Neerven *et al.* 1999; Wachholz *et al.* 2003]. One study even demonstrated sustained immunological changes of IgE-FAB for 2 years [Durham *et al.* 2012]. Furthermore, an inverse correlation has been found between symptom scores, rescue medication scores and IgE-FAB [Shamji *et al.* 2012; Schmid *et al.* 2014]. Unfortunately to date there are no data available on the possible relationship between IgE-FAB and responders *versus* nonresponders to AIT.

Concluding remarks

AR and asthma are common diseases that often coincide, while AR itself is an independent risk factor in the development of asthma. Disease burden has an impact on the quality of life and work productivity. When allergen avoidance and pharmacotherapy are not sufficient for disease control, AIT is advised under available guidelines. AIT is an effective, antigen-specific immune-modifying treatment that induces longterm tolerance. The mechanisms involved in AIT become clearer. Skewing from a Th2 response towards a more Th1 profile appears to play a key role in the mechanisms of AIT. IL-10 producing Tregs promote the development of sIgG4-producing plasma cells and sIgG4 competes with sIgE in allergen binding. A better understanding in the underlying

Table 1. An overview of biomarker responses in AIT studies.

Biomarkers	Allergens	Immunologic response	Number of studies	References
sIgG4	Grass	Increased	18	Didier *et al.* [2007]; Dahl *et al.* [2008]; Purohit *et al.* [2008]; Durham *et al.* [2010, 2012]; Scadding *et al.* [2010]; Blaiss *et al.* [2011]; James *et al.* [2011]; Nelson *et al.* [2011]; Klimek *et al.* [2012]; Shamji *et al.* [2012b, 2015]; Aasbjerg *et al.* [2014]; Ozdemira *et al.* [2014]; Keskin *et al.* [2006]; Amar *et al.* [2009]; Cox *et al.* [2012]; Wahn *et al.* [2012]
		Decreased	2	Ariano *et al.* [1999]; Zidarn *et al.* [2015]
		No change	2	Mosges *et al.* [2007]; Ott *et al.* [2009]
	HDM	Increased	8	Bousquet *et al.* [1999]; Lue *et al.* [2006]; Pham-Thi *et al.* [2007]; O'Hehir *et al.* [2009]; Zielen *et al.* [2010]; Keles *et al.* [2011]; Yukselen *et al.* [2012]; Gomez *et al.* [2015]
		No change	3	Cosmi *et al.* [2006]; Yukselen *et al.* [2012]; Wang *et al.* [2013]
IgE-BF	Grass	Increased	8	Dahl *et al.* [2008]; Durham *et al.* [2010]; Blaiss *et al.* [2011]; Nelson *et al.* [2011]; Durham *et al.* [2012]; Shamji *et al.* [2012b]; Aasbjerg *et al.* [2014]; Schmid *et al.* [2014]
	HDM	Increased	1	Corzo *et al.* [2014]
IgE-FAB	Grass	Increased	7	Scadding *et al.* [2010]; James *et al.* [2011]; Durham *et al.* [2012]; Shamji *et al.* [2012b, 2015]; Aasbjerg *et al.* [2014]; Schmid *et al.* [2014]
sIgE/tIgE	Grass	Increased	1	Di Lorenzo *et al.* [2009]
	HDM	Increased	2	Di Lorenzo *et al.* [2009]; Li *et al.* [2014]
		No change	1	Eifan *et al.* [2010]

AIT, allergen immunotherapy; HDM, house dust mite; Ig, immunoglobulin; IgE-BF, IgE blocking factor; IgE-FAB, IgE-facilitated antigen binding to B-cells; sIgE/tIgE, ratio of sIgE over total IgE; sIgG4, allergen specific IgG4.

mechanisms will eventually lead to novel treatment modalities as well as biomarkers for the follow up of AIT.

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References

Aalberse, R. and Schuurman, J. (2002) IgG4 breaking the rules. *Immunology* 105: 9–19.

Aasbjerg, K., Backer, V., Lund, G., Holm, J., Nielsen, N., Holse, M. *et al.* (2014) Immunological comparison of allergen immunotherapy tablet treatment and subcutaneous immunotherapy against grass allergy. *Clin Exp Allergy* 44: 417–428.

Akdis, C. and Akdis, M. (2015a) Advances in allergen immunotherapy: aiming for complete tolerance to allergens. *Sci Transl Med* 7: 280ps286.

Akdis, C. and Akdis, M. (2015b) Mechanisms of allergen-specific immunotherapy and immune tolerance to allergens. *World Allergy Organ J* 8: 17.

Akdis, M. and Akdis, C. (2014) Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens. *J Allergy Clin Immunol* 133: 621–631.

Amar, S., Harbeck, R., Sills, M., Silveira, L., O'Brien, H. and Nelson, H. (2009) Response to sublingual immunotherapy with grass pollen extract: monotherapy versus combination in a multiallergen extract. *J Allergy Clin Immunol* 124: 150–156.

Angelini, F., Pacciani, V., Corrente, S., Silenzi, R., Di Pede, A., Polito, A. *et al.* (2011) Dendritic cells modification during sublingual immunotherapy in children with allergic symptoms to house dust mites. *World J Pediatr* 7: 24–30.

Ariano, R., Kroon, A., Augeri, G., Canonica, G. and Passalacqua, G. (1999) Long-term treatment with allergoid immunotherapy with Parietaria. Clinical and

immunologic effects in a randomized, controlled trial. *Allergy* 54: 313–319.

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

Bahceciler, N., Arikan, C., Taylor, A., Akdis, M., Blaser, K., Barlan, I. *et al.* (2005) Impact of sublingual immunotherapy on specific antibody levels in asthmatic children allergic to house dust mites. *Int Arch Allergy Immunol* 136: 287–294.

Bauchau, V. and Durham, S. (2004) Prevalence and rate of diagnosis of allergic rhinitis in Europe. *Eur Respir J* 24: 758–764.

Bellinghausen, I., Brand, U., Steinbrink, K., Enk, A., Knop, J. and Saloga, J. (2001) Inhibition of human allergic T-cell responses by IL-10-treated dendritic cells: differences from hydrocortisone-treated dendritic cells. *J Allergy Clin Immunol* 108: 242–249.

Bernstein, J. (2010) Allergic and mixed rhinitis: epidemiology and natural history. *Allergy Asthma Proc* 31: 365–369.

Blaiss, M., Maloney, J., Nolte, H., Gawchik, S., Yao, R. and Skoner, D. (2011) Efficacy and safety of timothy grass allergy immunotherapy tablets in North American children and adolescents. *J Allergy Clin Immunol* 127: 64–71.

Bohle, B., Kinaciyan, T., Gerstmayr, M., Radakovics, A., Jahn-Schmid, B. and Ebner, C. (2007) Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. *J Allergy Clin Immunol* 120: 707–713.

Bousquet, J., Demoly, P. and Michel, F. (2001a) Specific immunotherapy in rhinitis and asthma. *Ann Allergy Asthma Immunol* 87(Suppl. 1): 38–42.

Bousquet, J., Khaltaev, N., Cruz, A., Denburg, J., Fokkens, W., Togias, A. *et al.* (2008) Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 63(Suppl. 86): 8–160.

Bousquet, J., Lockey, R. and Malling, H. (1998) Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 102: 558–562.

Bousquet, J., Scheinmann, P., Guinnepain, M., Perrin-Fayolle, M., Sauvaget, J., Tonnel, A. *et al.* (1999) Sublingual-swallow immunotherapy (SLIT) in patients with asthma due to house-dust mites: a double-blind, placebo-controlled study. *Allergy* 54: 249–260.

Bousquet, J., Schunemann, H., Samolinski, B., Demoly, P., Baena-Cagnani, C., Bachert, C. *et al.* (2012) Allergic Rhinitis and its Impact on Asthma (ARIA): achievements in 10 years and future needs. *J Allergy Clin Immunol* 130: 1049–1062.

Bousquet, J., Van Cauwenberge, P. and Khaltaev, N.Aria Workshop Group and World Health Organization (2001b) Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 108(Suppl. 5): S147–S334.

Braido, F., Sclifo, F., Ferrando, M. and Canonica, G. (2014) New therapies for allergic rhinitis. *Curr Allergy Asthma Rep* 14: 422.

Braunstahl, G. (2011) Chronic rhinosinusitis, nasal polyposis and asthma: the united airways concept reconsidered? *Clin Exp Allergy* 41: 1341–1343.

Brozek, J., Bousquet, J., Baena-Cagnani, C., Bonini, S., Canonica, G., Casale, T. *et al.* (2010) Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines: 2010 revision. *J Allergy Clin Immunol* 126: 466–476.

Burks, A., Calderon, M., Casale, T., Cox, L., Demoly, P., Jutel, M. *et al.* (2013) Update on allergy immunotherapy: American Academy of Allergy, Asthma & Immunology/European Academy of Allergy and Clinical Immunology/PRACTALL consensus report. *J Allergy Clin Immunol* 131: 1288–1296.

Calderon, M., Alves, B., Jacobson, M., Hurwitz, B., Sheikh, A. and Durham, S. (2007) Allergen injection immunotherapy for seasonal allergic rhinitis. *Cochrane Database Syst Rev* 1: CD001936.

Calderon, M., Casale, T., Cox, L., Akdis, C., Burks, A., Nelson, H. *et al.* (2013) Allergen immunotherapy: a new semantic framework from the European Academy of Allergy and Clinical Immunology/ American Academy of Allergy, Asthma and Immunology/PRACTALL consensus report. *Allergy* 68: 825–828.

Calderon, M., Penagos, M., Sheikh, A., Canonica, G. and Durham, S. (2011) Sublingual immunotherapy for treating allergic conjunctivitis. *Cochrane Database Syst Rev* 7: CD007685.

Canonica, G., Cox, L., Pawankar, R., Baena-Cagnani, C., Blaiss, M., Bonini, S. *et al.* (2014) Sublingual immunotherapy: World Allergy Organization position paper 2013 update. *World Allergy Organ J* 7: 6.

Ceuppens, J., Bullens, D., Kleinjans, H. and van der Werf, J. and PURETHAL Birch Efficacy Study Group. (2009) Immunotherapy with a modified birch pollen extract in allergic rhinoconjunctivitis: clinical and immunological effects. *Clin Exp Allergy* 39: 1903–1909.

Chelladurai, Y., Suarez-Cuervo, C., Erekosima, N., Kim, J., Ramanathan, M., Segal, J. *et al.* (2013)

Effectiveness of subcutaneous versus sublingual immunotherapy for the treatment of allergic rhinoconjunctivitis and asthma: a systematic review. *J Allergy Clin Immunol Pract* 1: 361–369.

Cockburn, I., Bailit, H., Berndt, E. and Finkelstein, S. (1999) Loss of work productivity due to illness and medical treatment. *J Occup Environ Med* 41: 948–953.

Compalati, E., Braido, F. and Canonica, G. (2014) An update on allergen immunotherapy and asthma. *Curr Opin Pulm Med* 20: 109–117.

Compalati, E., Ridolo, E., Passalacqua, G., Braido, F., Villa, E. and Canonica, G. (2010) The link between allergic rhinitis and asthma: the united airways disease. *Expert Rev Clin Immunol* 6: 413–423.

Cooke, R., Barnard, J., Hebald, S. and Stull, A. (1935) Serological evidence of immunity with coexisting sensitization in a type of human allergy (hay fever). *J Exp Med* 62: 733–750.

Corzo, J., Carrillo, T., Pedemonte, C., Martin, A., Hurtado, S., Dige, E. *et al.* (2014) Tolerability during double-blind randomized phase I trials with the house dust mite allergy immunotherapy tablet in adults and children. *J Investig Allergol Clin Immunol* 24: 154–161.

Cosmi, L., Santarlasci, V., Angeli, R., Liotta, F., Maggi, L., Frosali, F. *et al.* (2006) Sublingual immunotherapy with Dermatophagoides monomeric allergoid down-regulates allergen-specific immunoglobulin E and increases both interferon-gamma- and interleukin-10-production. *Clin Exp Allergy* 36: 261–272.

Cox, L., Nelson, H., Lockey, R., Calabria, C., Chacko, T., Finegold, I. *et al.* (2011) Allergen immunotherapy: a practice parameter third update. *J Allergy Clin Immunol* 127(Suppl. 1): S1–S55.

Cox, L., Casale, T., Nayak, A., Bernstein, D., Creticos, P., Ambroisine, L. *et al.* (2012) Clinical efficacy of 300IR 5-grass pollen sublingual tablet in a US study: the importance of allergen-specific serum IgE. *J Allergy Clin Immunol* 130: 1327–1334.

Cruz, A., Popov, T., Pawankar, R., Annesi-Maesano, I., Fokkens, W., Kemp, J. *et al.* (2007) Common characteristics of upper and lower airways in rhinitis and asthma: ARIA update, in collaboration with GA(2)LEN. *Allergy* 62(Suppl. 84): 1–41.

Dahl, R., Kapp, A., Colombo, G., De Monchy, J., Rak, S., Emminger, W. *et al.* (2008) Sublingual grass allergen tablet immunotherapy provides sustained clinical benefit with progressive immunologic changes over 2 years. *J Allergy Clin Immunol* 121: 512–518.

Di Bona, D., Plaia, A., Leto-Barone, M., La Piana, S. and Di Lorenzo, G. (2012) Efficacy of subcutaneous and sublingual immunotherapy with grass allergens for seasonal allergic rhinitis: a meta-analysis-based comparison. *J Allergy Clin Immunol* 130: 1097–1107.

Di Lorenzo, G., Mansueto, P., Pacor, M., Rizzo, M., Castello, F., Martinelli, N. *et al.* (2009) Evaluation of serum s-IgE/total IgE ratio in predicting clinical response to allergen-specific immunotherapy. *J Allergy Clin Immunol* 123: 1103–1110.

Didier, A., Malling, H., Worm, M., Horak, F., Jager, S., Montagut, A. *et al.* (2007) Optimal dose, efficacy, and safety of once-daily sublingual immunotherapy with a 5-grass pollen tablet for seasonal allergic rhinitis. *J Allergy Clin Immunol* 120: 1338–1345.

Dretzke, J., Meadows, A., Novielli, N., Huissoon, A., Fry-Smith, A. and Meads, C. (2013) Subcutaneous and sublingual immunotherapy for seasonal allergic rhinitis: a systematic review and indirect comparison. *J Allergy Clin Immunol* 131: 1361–1366.

Durham, S., Emminger, W., Kapp, A., Colombo, G., De Monchy, J., Rak, S. *et al.* (2010) Long-term clinical efficacy in grass pollen-induced rhinoconjunctivitis after treatment with SQ-standardized grass allergy immunotherapy tablet. *J Allergy Clin Immunol* 125: 131–138.

Durham, S., Emminger, W., Kapp, A., De Monchy, J., Rak, S., Scadding, G. *et al.* (2012) SQ-standardized sublingual grass immunotherapy: confirmation of disease modification 2 years after 3 years of treatment in a randomized trial. *J Allergy Clin Immunol* 129: 717–725.

Durham, S., Walker, S., Varga, E., Jacobson, M., O'brien, F., Noble, W. *et al.* (1999) Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 341: 468–475.

Ebo, D., Bridts, C., Mertens, C., Hagendorens, M., Stevens, W. and De Clerck, L. (2012) Analyzing histamine release by flow cytometry (HISTAFLOW): a novel instrument to study the degranulation patterns of basophils. *J Immunol Methods* 375: 30–38.

Eder, W., Ege, M. and Von Mutius, E. (2006) The asthma epidemic. *N Engl J Med* 355: 2226–2235.

Eifan, A., Akkoc, T., Yildiz, A., Keles, S., Ozdemir, C., Bahceciler, N. *et al.* (2010) Clinical efficacy and immunological mechanisms of sublingual and subcutaneous immunotherapy in asthmatic/rhinitis children sensitized to house dust mite: an open randomized controlled trial. *Clin Exp Allergy* 40: 922–932.

Faith, A., Richards, D., Verhoef, A., Lamb, J., Lee, T. and Hawrylowicz, C. (2003) Impaired secretion of interleukin-4 and interleukin-13 by allergen-specific T cells correlates with defective nuclear expression of NF-AT2 and jun B: relevance to immunotherapy. *Clin Exp Allergy* 33: 1209–1215.

Falcone, F., Knol, E. and Gibbs, B. (2011) The role of basophils in the pathogenesis of allergic disease. *Clin Exp Allergy* 41: 939–947.

Food and Drug Administration, H. (2008) International Conference on Harmonisation; Guidance on E15 Pharmacogenomics Definitions and Sample Coding; Availability. Notice. *Fed Regist* 73: 19074–19076.

Francis, J., James, L., Paraskevopoulos, G., Wong, C., Calderon, M., Durham, S. *et al.* (2008) Grass pollen immunotherapy: IL-10 induction and suppression of late responses precedes IgG4 inhibitory antibody activity. *J Allergy Clin Immunol* 121: 1120–1125.

Francis, J., Till, S. and Durham, S. (2003) Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol* 111: 1255–1261.

Fujimura, T. and Okamoto, Y. (2010) Antigen-specific immunotherapy against allergic rhinitis: the state of the art. *Allergol Int* 59: 21–31.

Gehlhar, K., Schlaak, M., Becker, W. and Bufe, A. (1999) Monitoring allergen immunotherapy of pollen-allergic patients: the ratio of allergen-specific IgG4 to IgG1 correlates with clinical outcome. *Clin Exp Allergy* 29: 497–506.

Gokmen, N., Ersoy, R., Gulbahar, O., Ardeniz, O., Sin, A., Unsel, M. *et al.* (2012) Desensitization effect of preseasonal seven-injection allergoid immunotherapy with olive pollen on basophil activation: the efficacy of olive pollen-specific preseasonal allergoid immunotherapy on basophils. *Int Arch Allergy Immunol* 159: 75–82.

Gomez, E., Fernandez, T., Dona, I., Rondon, C., Campo, P., Gomez, F. *et al.* (2015) Initial immunological changes as predictors for house dust mite immunotherapy response. *Clin Exp Allergy* 45: 1542–1553.

Guerra, S., Sherrill, D., Martinez, F. and Barbee, R. (2002) Rhinitis as an independent risk factor for adult-onset asthma. *J Allergy Clin Immunol* 109: 419–425.

Hellings, P., Muraro, A., Fokkens, W., Mullol, J., Bachert, C., Canonica, G. *et al.* (2015) A common language to assess allergic rhinitis control: results from a survey conducted during EAACI 2013 Congress. *Clin Transl Allergy* 5: 36.

Hurst, S., Muchamuel, T., Gorman, D., Gilbert, J., Clifford, T., Kwan, S. *et al.* (2002) New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *J Immunol* 169: 443–453.

Iliopoulos, O., Proud, D., Adkinson, N., Jr., Creticos, P., Norman, P., Kagey-Sobotka, A. *et al.* (1991) Effects of immunotherapy on the early, late, and rechallenge nasal reaction to provocation with allergen: changes in inflammatory mediators and cells. *J Allergy Clin Immunol* 87: 855–866.

Ishizaka, T., De Bernardo, R., Tomioka, H., Lichtenstein, L. and Ishizaka, K. (1972) Identification of basophil granulocytes as a site of allergic histamine release. *J Immunol* 108: 1000–1008.

Ito, T., Wang, Y., Duramad, O., Hori, T., Delespesse, G., Watanabe, N. *et al.* (2005) TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med* 202: 1213–1223.

James, L., Shamji, M., Walker, S., Wilson, D., Wachholz, P., Francis, J. *et al.* (2011) Long-term tolerance after allergen immunotherapy is accompanied by selective persistence of blocking antibodies. *J Allergy Clin Immunol* 127: 509–516.

Jutel, M. (2014) Allergen-specific immunotherapy in asthma. *Curr Treat Options Allergy* 1: 213–219.

Jutel, M., Agache, I., Bonini, S., Burks, A., Calderon, M., Canonica, W. *et al.* (2015) International consensus on allergy immunotherapy II: mechanisms, standardization, and pharmacoeconomics. *J Allergy Clin Immunol* 137: 358–368.

Jutel, M., Akdis, M., Budak, F., Aebischer-Casaulta, C., Wrzyszcz, M., Blaser, K. *et al.* (2003) IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 33: 1205–1214.

Jutel, M., Jaeger, L., Suck, R., Meyer, H., Fiebig, H. and Cromwell, O. (2005) Allergen-specific immunotherapy with recombinant grass pollen allergens. *J Allergy Clin Immunol* 116: 608–613.

Keles, S., Karakoc-Aydiner, E., Ozen, A., Izgi, A., Tevetoglu, A., Akkoc, T. *et al.* (2011) A novel approach in allergen-specific immunotherapy: combination of sublingual and subcutaneous routes. *J Allergy Clin Immunol* 128: 808–815.

Kepley, C., Cambier, J., Morel, P., Lujan, D., Ortega, E., Wilson, B. *et al.* (2000) Negative regulation of FcepsilonRI signaling by FcgammaRII costimulation in human blood basophils. *J Allergy Clin Immunol* 106: 337–348.

Keskin, O., Tuncer, A., Adalioglu, G., Sekerel, B., Sackesen, C. and Kalayci, O. (2006) The effects of grass pollen allergoid immunotherapy on clinical and immunological parameters in children with allergic rhinitis. *Pediatr Allergy Immunol* 17: 396–407.

Kirmaz, C., Ozenturk Kirgiz, O., Bayrak, P., Yilmaz, O., Vatansever, S., Ozbilgin, K. *et al.* (2011) Effects of allergen-specific immunotherapy on functions of

helper and regulatory T cells in patients with seasonal allergic rhinitis. *Eur Cytokine Netw* 22: 15–23.

Klimek, L., Schendzielorz, P., Pinol, R. and Pfaar, O. (2012) Specific subcutaneous immunotherapy with recombinant grass pollen allergens: first randomized dose-ranging safety study. *Clin Exp Allergy* 42: 936–945.

La Rosa, M., Ranno, C., Andre, C., Carat, F., Tosca, M. and Canonica, G. (1999) Double-blind placebo-controlled evaluation of sublingual-swallow immunotherapy with standardized Parietaria judaica extract in children with allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 104: 425–432.

Lalek, N., Kosnik, M., Silar, M. and Korosec, P. (2010) Immunoglobulin G-dependent changes in basophil allergen threshold sensitivity during birch pollen immunotherapy. *Clin Exp Allergy* 40: 1186–1193.

Lao-Araya, M., Steveling, E., Scadding, G., Durham, S. and Shamji, M. (2014) Seasonal increases in peripheral innate lymphoid type 2 cells are inhibited by subcutaneous grass pollen immunotherapy. *J Allergy Clin Immunol* 134: 1193–1195.

Li, Q., Li, M., Yue, W., Zhou, J., Li, R., Lin, J. *et al.* (2014) Predictive factors for clinical response to allergy immunotherapy in children with asthma and rhinitis. *Int Arch Allergy Immunol* 164: 210–217.

Lichtenstein, L., Norman, P. and Winkenwerder, W. (1968) Antibody response following immunotherapy in ragweed hay fever: allpyral versus whole ragweed extract. *J Allergy* 41: 49–57.

Lima, M., Wilson, D., Pitkin, L., Roberts, A., Nouri-Aria, K., Jacobson, M. *et al.* (2002) Grass pollen sublingual immunotherapy for seasonal rhinoconjunctivitis: a randomized controlled trial. *Clin Exp Allergy* 32: 507–514.

Lue, K., Lin, Y., Sun, H., Lu, K., Hsieh, J. and Chou, M. (2006) Clinical and immunologic effects of sublingual immunotherapy in asthmatic children sensitized to mites: a double-blind, randomized, placebo-controlled study. *Pediatr Allergy Immunol* 17: 408–415.

Macglashan, D., Jr. (2010) Expression of CD203c and CD63 in human basophils: relationship to differential regulation of piecemeal and anaphylactic degranulation processes. *Clin Exp Allergy* 40: 1365–1377.

Malling, H. and Bousquet, J. (2008) Subcutaneous immunotherapy for allergic rhinoconjunctivitis, allergic asthma, and prevention of allergic diseases. *Clin Allergy Immunol* 21: 343–358.

Martinez, F. and Vercelli, D. (2013) Asthma. *Lancet* 382: 1360–1372.

Mitra, M., Kandalam, M., Harilal, A., Verma, R., Krishnan, U., Swaminathan, S. *et al.* (2012) EpCAM is a putative stem marker in retinoblastoma and an effective target for T-cell-mediated immunotherapy. *Mol Vis* 18: 290–308.

Mosges, R., Bruning, H., Hessler, H., Gotz, G. and Knaussmann, H. (2007) Sublingual immunotherapy in pollen-induced seasonal rhinitis and conjunctivitis: a randomized controlled trial. *Acta Dermatovenerol Alp Pannonica Adriat* 16: 143–148.

Moverare, R., Elfman, L., Vesterinen, E., Metso, T. and Haahtela, T. (2002) Development of new IgE specificities to allergenic components in birch pollen extract during specific immunotherapy studied with immunoblotting and Pharmacia CAP System. *Allergy* 57: 423–430.

Nanda, A., O'connor, M., Anand, M., Dreskin, S., Zhang, L., Hines, B. *et al.* (2004) Dose dependence and time course of the immunologic response to administration of standardized cat allergen extract. *J Allergy Clin Immunol* 114: 1339–1344.

Nelson, H., Nolte, H., Creticos, P., Maloney, J., Wu, J. and Bernstein, D. (2011) Efficacy and safety of timothy grass allergy immunotherapy tablet treatment in North American adults. *J Allergy Clin Immunol* 127: 72–80.

Nish, W., Charlesworth, E., Davis, T., Whisman, B., Valtier, S., Charlesworth, M. *et al.* (1994) The effect of immunotherapy on the cutaneous late phase response to antigen. *J Allergy Clin Immunol* 93: 484–493.

Nopp, A., Cardell, L., Johansson, S. and Oman, H. (2009) CD-sens: a biological measure of immunological changes stimulated by ASIT. *Allergy* 64: 811–814.

Nouri-Aria, K., Wachholz, P., Francis, J., Jacobson, M., Walker, S., Wilcock, L. *et al.* (2004) Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J Immunol* 172: 3252–3259.

Novak, N., Bieber, T. and Allam, J. (2011) Immunological mechanisms of sublingual allergen-specific immunotherapy. *Allergy* 66: 733–739.

O'Hehir, R., Gardner, L., De Leon, M., Hales, B., Biondo, M., Douglass, J. *et al.* (2009) House dust mite sublingual immunotherapy: the role for transforming growth factor-beta and functional regulatory T cells. *Am J Respir Crit Care Med* 180: 936–947.

Ott, H., Sieber, J., Brehler, R., Folster-Holst, R., Kapp, A., Klimek, L. *et al.* (2009) Efficacy of grass pollen sublingual immunotherapy for three consecutive seasons and after cessation of treatment: the ECRIT study. *Allergy* 64: 1394–1401.

Ozdemira, S., Sin, B., Gulogu, D., Ikinciogullari, A., Gencturk, Z. and Misirligil, Z. (2014) Short-term preseasonal immunotherapy: is early clinical efficacy related to the basophil response? *Int Arch Allergy Immunol* 164: 237–245.

Pfaar, O., Demoly, P., Gerth Van Wijk, R., Bonini, S., Bousquet, J., Canonica, G. *et al.* (2014) Recommendations for the standardization of clinical outcomes used in allergen immunotherapy trials for allergic rhinoconjunctivitis: an EAACI Position Paper. *Allergy* 69: 854–867.

Pham-Thi, N., Scheinmann, P., Fadel, R., Combebias, A. and Andre, C. (2007) Assessment of sublingual immunotherapy efficacy in children with house dust mite-induced allergic asthma optimally controlled by pharmacologic treatment and mite-avoidance measures. *Pediatr Allergy Immunol* 18: 47–57.

Pienkowski, M., Norman, P. and Lichtenstein, L. (1985) Suppression of late-phase skin reactions by immunotherapy with ragweed extract. *J Allergy Clin Immunol* 76: 729–734.

Pilette, C., Nouri-Aria, K., Jacobson, M., Wilcock, L., Detry, B., Walker, S. *et al.* (2007) Grass pollen immunotherapy induces an allergen-specific IgA2 antibody response associated with mucosal TGF-beta expression. *J Immunol* 178: 4658–4666.

Pitsios, C., Demoly, P., Bilo, M., Gerth Van Wijk, R., Pfaar, O., Sturm, G. *et al.* (2015) Clinical contraindications to allergen immunotherapy: an EAACI position paper. *Allergy* 70(8): 897–909.

Platts-Mills, T., Von Maur, R., Ishizaka, K., Norman, P. and Lichtenstein, L. (1976) IgA and IgG anti-ragweed antibodies in nasal secretions. Quantitative measurements of antibodies and correlation with inhibition of histamine release. *J Clin Invest* 57: 1041–1050.

Puggioni, F., Durham, S. and Francis, J. (2005) Monophosphoryl lipid A (MPL) promotes allergen-induced immune deviation in favour of Th1 responses. *Allergy* 60: 678–684.

Purohit, A., Niederberger, V., Kronqvist, M., Horak, F., Gronneberg, R., Suck, R. *et al.* (2008) Clinical effects of immunotherapy with genetically modified recombinant birch pollen Bet v 1 derivatives. *Clin Exp Allergy* 38: 1514–1525.

Radulovic, S., Jacobson, M., Durham, S. and Nouri-Aria, K. (2008) Grass pollen immunotherapy induces Foxp3-expressing CD4+ CD25+ cells in the nasal mucosa. *J Allergy Clin Immunol* 121: 1467–1472.

Reddel, H., Bateman, E., Becker, A., Boulet, L., Cruz, A., Drazen, J. *et al.* (2015a) A summary of the new GINA strategy: a roadmap to asthma control. *Eur Respir J* 46(3): 622–639.

Reddel, H. and Levy, M. Global Initiative for Asthma Scientific Committee and Dissemination and Implementation Committee (2015b) The GINA asthma strategy report: what's new for primary care? *NPJ Prim Care Respir Med* 25: 15050.

Reisinger, J., Horak, F., Pauli, G., Van Hage, M., Cromwell, O., Konig, F. *et al.* (2005) Allergen-specific nasal IgG antibodies induced by vaccination with genetically modified allergens are associated with reduced nasal allergen sensitivity. *J Allergy Clin Immunol* 116: 347–354.

Rispens, T., Ooijevaar-De Heer, P., Bende, O. and Aalberse, R. (2011) Mechanism of immunoglobulin G4 Fab-arm exchange. *J Am Chem Soc* 133: 10302–10311.

Rolinck-Werninghaus, C., Kopp, M., Liebke, C., Lange, J., Wahn, U. and Niggemann, B. (2005) Lack of detectable alterations in immune responses during sublingual immunotherapy in children with seasonal allergic rhinoconjunctivitis to grass pollen. *Int Arch Allergy Immunol* 136: 134–141.

Rolland, J., Gardner, L. and O'Hehir, R. (2010) Functional regulatory T cells and allergen immunotherapy. *Curr Opin Allergy Clin Immunol* 10: 559–566.

Saleem, N., Chaker, A., Zissler, U., Schmidt-Weber, C., Durham, S. and Shamji, M. (2013) Local nasal 'protective' immunoglobulin G4 (IgG4) responses in nasal fluid following grass pollen sublingual immunotherapy. *J Allergy Clin Immunol* 2: AB202.

Scadding, G. (2015) Optimal management of allergic rhinitis. *Arch Dis Child* 100: 576–582.

Scadding, G., Eifan, A., Lao-Araya, M., Penagos, M., Poon, S., Steveling, E. *et al.* (2015) Effect of grass pollen immunotherapy on clinical and local immune response to nasal allergen challenge. *Allergy* 70: 689–696.

Scadding, G., Shamji, M., Jacobson, M., Lee, D., Wilson, D., Lima, M. *et al.* (2010) 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* 40: 598–606.

Schmid, J., Wurtzen, P., Dahl, R. and Hoffmann, H. (2014) Early improvement in basophil sensitivity predicts symptom relief with grass pollen immunotherapy. *J Allergy Clin Immunol* 134: 741–744.

Schmitz, J., Owyang, A., Oldham, E., Song, Y., Murphy, E., Mcclanahan, T. *et al.* (2005) 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* 23: 479–490.

Schroeder, J., Kagey-Sobotka, A. and Lichtenstein, L. (1995) The role of the basophil in allergic inflammation. *Allergy* 50: 463–472.

Shamji, M. and Durham, S. (2011) Mechanisms of immunotherapy to aeroallergens. *Clin Exp Allergy* 41: 1235–1246.

Shamji, M., James, L. and Durham, S. (2011) Serum immunologic markers for monitoring allergen-specific immunotherapy. *Immunol Allergy Clin North Am* 31: 311–323.

Shamji, M., Achkova, D., Layhadi, J., Perera-Webb, A., Scadding, G., Khan, S. *et al.* (2012a) *IL-35 producing regulatory T cells modulate grass pollen-specific Th2 responses and are induced following sublingual grass pollen immunotherapy*. Hoboken, NJ: Wiley-Blackwell.

Shamji, M., Francis, J., Wurtzen, P., Lund, K., Durham, S. and Till, S. (2013a) Cell-free detection of allergen-IgE cross-linking with immobilized phase CD23: inhibition by blocking antibody responses after immunotherapy. *J Allergy Clin Immunol* 132: 1003–1005.

Shamji, M., Layhadi, J., Perera-Web, A., Scadding, G. and Durham, S. (2013b) Il-35+ regulatory T cells suppress grass pollen-driven Th2 responses and are induced following grass pollen-specific sublingual immunotherapy. *J Allergy Clin Immunol* 2: AB146.

Shamji, M., Layhadi, J., Scadding, G., Cheung, D., Calderon, M., Turka, L. *et al.* (2015) Basophil expression of diamine oxidase: a novel biomarker of allergen immunotherapy response. *J Allergy Clin Immunol* 135: 913–921.

Shamji, M., Ljorring, C., Francis, J., Calderon, M., Larche, M., Kimber, I. *et al.* (2012b) Functional rather than immunoreactive levels of IgG4 correlate closely with clinical response to grass pollen immunotherapy. *Allergy* 67: 217–226.

Shamji, M., Saleem, N., Nicholson, J., Schmidt-Weber, C., Chaker, A. and Durham, S. (2013c) *Local nasal antiinflammatory igg4 responses are induced following grass pollen sublingual and subcutaneous immunotherapy*. Hoboken, NJ: Wiley-Blackwell.

Simons, F. (1996) Learning impairment and allergic rhinitis. *Allergy Asthma Proc* 17: 185–189.

Soumelis, V., Reche, P., Kanzler, H., Yuan, W., Edward, G., Homey, B. *et al.* (2002) Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 3: 673–680.

Sullivan, P., Ghushchyan, V., Slejko, J., Belozeroff, V., Globe, D. and Lin, S. (2011) The burden of adult asthma in the United States: evidence from the Medical Expenditure Panel Survey. *J Allergy Clin Immunol* 127: 363–369.

Troise, C., Voltolini, S., Canessa, A., Pecora, S. and Negrini, A. (1995) Sublingual immunotherapy in Parietaria pollen-induced rhinitis: a double-blind study. *J Investig Allergol Clin Immunol* 5: 25–30.

Van Der Neut Kolfschoten, M., Schuurman, J., Losen, M., Bleeker, W., Martinez-Martinez, P., Vermeulen, E. *et al.* (2007) Anti-inflammatory activity of human IgG4 antibodies by dynamic fab arm exchange. *Science* 317: 1554–1557.

Van Neerven, R., Wikborg, T., Lund, G., Jacobsen, B., Brinch-Nielsen, A., Arnved, J. *et al.* (1999) Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4+ T cells by inhibiting serum-IgE-facilitated allergen presentation. *J Immunol* 163: 2944–2952.

Van Overtvelt, L., Baron-Bodo, V., Horiot, S., Moussu, H., Ricarte, C., Horak, F. *et al.* (2011) Changes in basophil activation during grass-pollen sublingual immunotherapy do not correlate with clinical efficacy. *Allergy* 66: 1530–1537.

Varney, V., Hamid, Q., Gaga, M., Ying, S., Jacobson, M., Frew, A. *et al.* (1993) Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J Clin Invest* 92: 644–651.

Wachholz, P., Nouri-Aria, K., Wilson, D., Walker, S., Verhoef, A., Till, S. *et al.* (2002) Grass pollen immunotherapy for hayfever is associated with increases in local nasal but not peripheral Th1:Th2 cytokine ratios. *Immunology* 105: 56–62.

Wachholz, P., Soni, N., Till, S. and Durham, S. (2003) Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy. *J Allergy Clin Immunol* 112: 915–922.

Wahn, U., Klimek, L., Ploszczuk, A., Adelt, T., Sandner, B., Trebas-Pietras, E. *et al.* (2012) High-dose sublingual immunotherapy with single-dose aqueous grass pollen extract in children is effective and safe: a double-blind, placebo-controlled study. *J Allergy Clin Immunol* 130: 886–893.

Wang, D., Chen, L., Cheng, L., Li, K., Yuan, H., Lu, J. *et al.* (2013) Fast onset of action of sublingual immunotherapy in house dust mite-induced allergic rhinitis: a multicenter, randomized, double-blind, placebo-controlled trial. *Laryngoscope* 123: 1334–1340.

Warner, J., Price, J., Soothill, J. and Hey, E. (1978) Controlled trial of hyposensitisation to Dermatophagoides pteronyssinus in children with asthma. *Lancet* 2: 912–915.

Wheatley, L. and Togias, A. (2015) Clinical practice. Allergic rhinitis. *N Engl J Med* 372: 456–463.

Wurtzen, P., Lund, G., Lund, K., Arvidsson, M., Rak, S. and Ipsen, H. (2008) 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* 38: 1290–1301.

Yukselen, A., Kendirli, S., Yilmaz, M., Altintas, D. and Karakoc, G. (2012) Effect of one-year subcutaneous and sublingual immunotherapy on clinical and laboratory parameters in children with rhinitis and asthma: a randomized, placebo-controlled, double-blind, double-dummy study. *Int Arch Allergy Immunol* 157: 288–298.

Zidarn, M., Kosnik, M., Silar, M., Bajrovic, N. and Korosec, P. (2015) Sustained effect of grass pollen subcutaneous immunotherapy on suppression of allergen-specific basophil response; a real-life, nonrandomized controlled study. *Allergy* 70: 547–555.

Zielen, S., Kardos, P. and Madonini, E. (2010) Steroid-sparing effects with allergen-specific immunotherapy in children with asthma: a randomized controlled trial. *J Allergy Clin Immunol* 126: 942–949.

Zimmer, A., Bouley, J., Le Mignon, M., Pliquet, E., Horiot, S., Turfkruyer, M. *et al*. (2012) A regulatory dendritic cell signature correlates with the clinical efficacy of allergen-specific sublingual immunotherapy. *J Allergy Clin Immunol* 129: 1020–1030.