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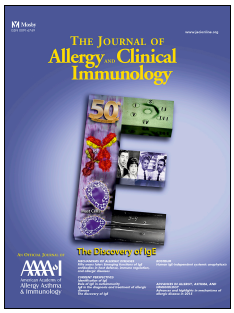

Advances and Highlights in Allergen Immunotherapy: On the way to sustained clinical and immunologic tolerance

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Advances and Highlights in Allergen Immunotherapy: On the way to sustained clinical and immunologic tolerance

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Key words: Allergic rhinitis, asthma, allergen-specific immunotherapy, mechanisms, meta analysis, clinical trials, immune tolerance, food allergy

Abbreviations

AIT	allergen immunotherapy
AR	allergic rhinitis
ATS	American Thoracic Society
DC	dendritic cell
EPIT	epicutaneous immunotherapy
ERS	European Respiratory Society
FA	food allergy
HDM	house dust mite
ICS	inhaled glucocorticosteroids
ILIT	intra lymph node immunotherapy
IT	immunotherapy
OIT	oral immunotherapy
RCT	randomized controlled trial
SCIT	subcutaneous immunotherapy
SLIT	sublingual immunotherapy
SAR	systemic adverse reactions
WAO	World Allergy Organization

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Abstract

Allergen immunotherapy (AIT) is an effective treatment strategy for allergic diseases and has been used for more than 100 years. In recent years, however, the expectations on concepts, conduct, statistical evaluation and reporting have been significantly developed. Products have undergone dose-response and confirmative studies in adults and children to provide evidence for the optimal dosage, safety and efficacy of AIT vaccines using subcutaneous and sublingual delivery pathways in large patient cohorts, ensuring solid conclusions to be drawn from them for the advantage of patients and societies alike. Those standards should be followed today, and products answering to them should be preferred over others lacking optimization and proof of efficacy and safety. Molecular and cellular mechanisms of AIT include early mast cell and basophil desensitization effects, regulation of T and B cell responses, regulation of IgE and IgG4 production and inhibition of responses from eosinophils, mast cells and basophils in the affected tissues. There were many developments to improve vaccination strategies, demonstration of new molecules involved in molecular mechanisms and demonstration of new biomarkers for AIT during the last few years. The combination of probiotics, vitamins and biologicals with AIT are highlighting current advances. Development of allergoids, recombinant and hypoallergenic vaccines to skew the immune response from IgE to IgG4 and regulation of dendritic cell, mast cell, basophil, innate lymphoid cell, T and B cell responses to allergens are also discussed in detail.

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Introduction

In this review, a number of key developments and publications in the field of allergen immunotherapy (AIT) published recently will be discussed. Large AIT studies with state-of-the-art protocols, evaluations and reporting for allergic rhinitis (AR) and allergic asthma have been performed, which considerably increase the efficacy of specific AIT products and develop the field into a future of evidence-based treatment. [1] Both subcutaneous (SCIT) and sublingual immunotherapies (SLIT) have been established as viable and safe procedures if administered with the correct products.[2] The international consensus consortium on allergen immunotherapy has further encouraged researchers in the field to contribute to the generation of hypoallergenic recombinant allergen derivatives and immunogenic peptides, developing new adjuvants and stimulators of the innate immune response, fusion of allergens with immune modifiers and peptide carrier proteins for efficient vaccination, and new routes of vaccine administration. [3] This review highlights new studies performed on efficacy and safety of SCIT and SLIT to allow for best practices in AIT treatments, and current developments in mechanisms of immune tolerance to allergens using AIT as the best human in vivo model to study immune regulation.

Evidence in allergen immunotherapy: meta-analyses and guidelines

Universal standardization of allergen extracts is a prerequisite to develop efficient tools for the diagnosis and therapy of atopic disease. Allergen extracts are being currently standardized using established methods to control their potency, composition and stability of the major allergen, however there is no universally accepted methodology that enables the comparison of products of different companies that spans all AIT vaccines.[3] In addition, state-of-the art studies with AIT products should provide evidence for efficacy and tolerability. European directives classified allergen products as medicinal products, providing specifications for allergen products in both diagnostics and AIT. Under these regulations, allergen products require a market authorization similar to medicinal drugs, which includes dose-finding and confirmation of efficacy and tolerability in Phase III studies. As allergen products are meant to modulate immune tolerance in the patient, such prerequisites seem more than justified to assure an efficacious treatment. Although a large number of national and international guidelines for AIT have been developed [2], they fail to base their recommendations on established doses per product, but rather use meta-analyses of registered and non-registered

products, for which dose-finding studies may not even be available. As a consequence of the heterogeneity of meta-analyses, these guidelines limit their conclusions to general statements on AIT, mostly based on the application route; they can by no means be of support to select an efficacious product for a specific patient. Obviously, claims from meta-analyses or from specific AIT products cannot be used for whatever other product; there is no "class effect" due to the heterogeneity in composition and dosing of individual products. [4] This gets specifically obvious for AIT in children, claims on asthma prevention etc., but also excludes claims on mixtures unless optimal dosing is used per allergen in the mixture. This, however, meets obvious limitations regarding safety. A recent World Allergy Organization (WAO) statement indicates the need for product-specific evidence-based AIT. [5]

In the past, we were used to honour meta-analyses for their high scientific status; however, there is some criticism on flaws in technique, but also wrong conclusions from meta-analyses. [6-8] Meta-analyses should provide the highest level of evidence for the efficacy of a medical treatment or intervention. In recent years there has been an overflow of meta-analyses on AIT with contrasting results that may generate confusion among physicians. [8] Flaws may result from incorrect selection of trials, inappropriate use of evaluation parameters for the analysis, bias towards unpublished studies, unsuitable analyses and over-interpretation of the results. The most obvious error results from the selection of different products into one meta-analysis, which necessarily results in heterogeneity. It is clear that a meta-analysis of several small studies with various products does not predict the result of a state-of-art large study with a dose-optimized standardized product. The gold standard for AIT is a product for which a dose-ranging study has been performed and larger phase-III studies are available for adults and children over at least 1 year treatment, and optimally a study in adults over 3 years and two years follow-up for claims on long-term efficacy and disease modification. [5, 9] One such study tested grass pollen products on moderate to severe AR patients undergoing grass pollen SLIT and SCIT for 2 years, with a furthermore 3-year follow-up, and found no significant improvement of nasal response over placebo control at the 3rd year. However, grass pollen specific IgE levels were decreased after 2 and 3 years in patients undergone SCIT.[10]

Recent advances in allergen immunotherapy for allergic rhinoconjunctivitis and asthma

House dust mites (HDMs) are major perennial allergen sources and a significant cause of AR and allergic asthma; a recent overview lined out that 65 to 130 million people worldwide may suffer from HDM allergy, with up to 50% of asthmatic patients being sensitized. [11] HDM

sensitizations are more frequently associated with both, rhinitis and asthma than any other frequent allergen. Still, HDM allergies remain undiagnosed and undertreated. As a consequence, the need for treatment of HDM-induced asthma and AR is high; so far, however, there was a deficit in adequately studied AIT products for HDM allergic subjects. Furthermore, international guidelines restricted AIT application in uncontrolled asthma patients. [4]

Based on dose-finding studies in a challenge chamber, strong evidence for the efficacy, tolerability and safety of HDM sublingual immunotherapy tablets has accumulated in classical state-of-the-art field studies during the past two years. [12, 13] Results from several large, multicenter, double-blind placebo controlled randomized trials (RCT) support the efficacy of HDM sublingual tablets in both adults and adolescents (> 12 years) with AR **(Online Repository Table 1**). [14-17] Moreover, a large, multicentre randomized controlled trial (RCT) has studied for the first time the effect of HDM sublingual tablets in adults (n=834) with HDM allergy-related asthma not well controlled by inhaled corticosteroids (ICS) or combination treatment. The study showed a significantly reduced risk for moderate or severe asthma exacerbations, as defined by ATS-ERS criteria, during 6 months period of ICS reduction. Treatment-related adverse events were common, but were primarily mild to moderate local reactions. Further studies are needed to evaluate long-term efficacy and safety of HDM AIT in asthma. [18]

Compared to the accumulating evidence in HDM tablets, there is only one recent publication of an RCT evaluating the efficacy of HDM subcutaneous immunotherapy. [19] This dose-finding study demonstrated significant improvement in the clinical response to a titrated nasal provocation test after 12 months treatment with HDM allergoid SCIT in the three higher dose groups. A post hoc analysis of the combined symptom and medication scores in a subgroup of patients with a higher score at the start of the study showed a significant treatment effect in the two higher dosages for the last 8 weeks of study, potentially translating the efficacy observed in the nasal challenge to clinics. However, due to a higher incidence of adverse events in the highest dose group, the study favored the intermediate doses for further clinical use. This study demonstrates that both, efficacy and safety have to be taken into account when developing optimal AIT drugs.

It has previously been suggested that allergen immunotherapy might prevent the onset of new sensitizations and the manifestation of asthma in patients with AR. A prospective,

randomized, double-blind, placebo-controlled proof-of-concept study involved 111 infants (aged 5 to 9 months) at high hereditary risk to atopy (≥ 2 first-degree relatives with allergic disease) with negative skin prick tests to common food- and aeroallergens at randomization. The study revealed that twice daily administration for 12 months of high dose HDM extract oral immunotherapy (OIT) solution was associated with a significant reduction in sensitization to any common allergen compared to placebo; however, no significant preventive effect was observed on HDM sensitization or allergy-related symptoms. Thus, it remains unclear whether early intervention in high-risk children is a valuable option. [20]

For the first time, however, evidence for a reduction in the development of asthma in patients with AR, diagnosed according to International Statistical Classification of Diseases, 10th Edition, by allergen immunotherapy has been provided in a "real-life setting", large, retrospective, cohort study using routine health care data from German National Health Insurance beneficiaries.[21] The cohort consisted of 118,754 patients with allergic rhinoconjunctivitis (ARC), but without asthma at inclusion, who had not received AIT before. The study revealed a significantly reduced risk of incident asthma for 5 years in patients exposed to AIT compared to those receiving no AIT (risk reduction by 40%). Sensitivity analyses suggested significant preventive effects of subcutaneous immunotherapy with native (non-allergoid) allergens.

Advances in allergen immunotherapy routes and preparations

Conventional subcutaneous immunotherapy is associated with some disadvantages, including the need for frequent injections over minimally 3 years, with the need for visits to the doctor's office, and the risk for adverse events including life-threatening anaphylaxis in few cases. Sublingual immunotherapy has emerged as an alternative user-friendly approach, allowing self-administration at home, at the same time reducing the risk of severe systemic reactions. However, also SLIT requires daily intake for three years, challenging the adherence of the patients to the treatment. As a consequence, novel AIT approaches are constantly in development (**Table 1**).

These approaches aim to improve safety and patient convenience, and at the same time to preserve or even improve efficacy. [22] Strategies include both, alternative routes of administration (including intradermal, epicutaneous, intralymphatic, oral or nasal administration) and alterations of the allergens (including allergoids, purified recombinant allergens, recombinant hypoallergenic allergens, and allergen peptides) (reviewed in 1).

Finally, the safety and efficacy of AIT may be improved when combined with other innovative treatments (such as monoclonal antibodies to IgE or T_H2 cytokines). Intradermal injections and epicutaneous applications using allergen patches have been proposed, but have not convinced so far. Repeated intradermal administrations of very low doses of grass pollen allergen extract have previously been associated with suppressed allergen skin late phase responses [23]; however, a recent RCT with pre-seasonal intradermal grass AIT [24] rather showed an increase in responsiveness and failed to show beneficial effects on allergic symptoms. Efficacy of epicutaneous AIT for treatment of grass-pollen-induced AR was supported previously by two RCTs [25, 26], but a recent trial performed by the same group [27] confirmed significant symptom improvement compared to placebo in the year of treatment, but not the year after discontinuation. Hence, a single course of pre-seasonal epicutaneous AIT was not associated with long-term efficacy.

Intralymphatic AIT (ILIT) involves a small number of injections of low doses of allergen directly into a lymph node. Efficacy and safety has been suggested for pollen-induced AR [28] and cat-induced AR. [29] An additional small cohort of patients with grass- or birch-pollen-induced AR [30] also showed an improved global evaluation of seasonal symptoms. In conclusion, further assessment in well-designed, large-scale RCTs is needed before ILIT is ready for clinical use.[31]

Investigations on allergen content and characterization of drug products used for clinical trials for OIT has been limited so far. One study showed that peanut flour contains major peanut allergens Ara h 1 and Ara h 2 in complete form and can be a viable product for peanut OIT with its low bacterial content and long shelf life.[32]

In recent years, two types of T cell epitope-based allergen peptides have been developed and assessed in RCTs: short T cell epitope peptides (also named 'Synthetic peptide immuno-regulatory epitopes', SPIREs) and longer Contiguous Overlapping Peptides (COPs). [33] Previous trials with cat [34], house dust mite [35] and grass [36] SPIRE peptide immunotherapy were encouraging. A more recent dose-finding Phase II trial evaluating the effect of grass peptide AIT showed significant improvement of rhinoconjunctivitis symptoms to grass allergen challenge with one of three tested dosing schemes. [37] However, the results of the Phase III trial with cat peptide AIT and the Phase IIb trial with HDM peptide AIT did not achieve clinical endpoints. [38, 39]

In the COP-based approach, all possible T cell epitopes of the target allergen are included in a small set of long synthetic peptides that are unable to bind IgE. Good clinical tolerability of Bet v 1-derived COP-based SCIT was shown in a previous Phase I/IIa clinical trial in patients with birch-pollen-induced AR, and clinical efficacy compared to placebo was demonstrated more recently in a Phase IIb trial with the lower of two dosing schemes. [40] In addition, a hypoallergenic B cell epitope-based peptide vaccine has been developed for grass pollen allergy. The vaccine lacks IgE reactivity and has maximally reduced allergen-specific T cell epitopes, aiming to avoid IgE-mediated early and T-cell mediated late phase adverse events. [41] Both safety and efficacy of this novel vaccine have been investigated in a Phase II trial [42]; a single course of three monthly subcutaneous injections was effective in reducing allergen-induced symptoms vs. baseline in the setting of an exposure chamber trial. No systemic immediate-type events and only a few grade 1 systemic late phase reactions occurred. Further trials are needed to establish the potential of this novel B cell epitope-based vaccine.

Finally, a small single-center RCT evaluated the combined approach of suboptimal grass SCIT with monoclonal anti-IL4 treatment. Although anti–IL-4 treatment was effective in modulating T_H2 memory, the study did not reveal additional benefit of combined treatment with anti-IL-4 over treatment with suboptimal grass SCIT alone on the allergen-induced cutaneous late-phase response. [43]

Recent advances in allergen immunotherapy for food allergy and atopic dermatitis

An increasing prevalence of food allergy in children and the observation that it takes longer than previously thought to outgrow food allergies [44] increases the need for novel treatments for food allergies other than avoidance, which remains the standard of care. [45] Different routes of allergen immunotherapy have been investigated, including oral immunotherapy (OIT), epicutaneous immunotherapy (EPIT), SLIT, and SCIT approaches. The recent publications of RCTs are summarized in **Table 2.** [46-55]

It is important to recognize the great heterogeneity of the trials in food allergy. [49] Dosing schemes, treatment duration and primary outcomes vary highly between trials, and included numbers of patients are rather small. Nevertheless, there is increasing evidence that desensitization to food allergens with AIT may be effective at least for the protection against accidental exposure. Two dose-finding RCTs have studied the efficacy of peanut EPIT using an allergen patch delivery system [50, 51] and revealed a modest but significant treatment effect

compared to placebo after 52 weeks of treatment. The treatment was found to be safe and well tolerated. Peanut EPIT is currently being assessed in a Phase III trial.

A trial in young children aged 9 to 36 months at randomization specifically aimed to evaluate safety, efficacy and feasibility of early peanut OIT with two doses. [52] In both treatment arms, a high proportion of children reached sustained unresponsiveness 4 weeks after treatment discontinuation. OIT treatment on pre-school children had a favourable safety profile. [52] Peanut OIT later in life showed a higher efficacy for treatment of peanut allergy compared to SLIT, but higher frequency of adverse reactions have been reported as a disadvantage.[47] The combination of food AIT with Omalizumab may further increase tolerability, efficacy, and may reduce the time needed for up-dosing, as shown for cow's milk and peanut allergy. [53, 54] The use of hydrolysed preparations for egg allergy may be another approach to increase safety [55]; larger RCTs will be needed to further assess these novel treatments.

To date, there is no consistent evidence supporting the effectiveness of AIT for the treatment of atopic dermatitis. Despite the demand for high-quality research to further evaluate the role of SIT in atopic dermatitis, [56-58] no new RCTs have been published recently.

Adverse events and safety

A European survey on systemic adverse reactions (SAR) aimed to monitor the real-life situation by asking physicians in 3 countries, 94% allergists, about adverse events in patients on AIT for pollen, alternaria and animal dander. [59] A total of 4316 patients, more than 50% with asthma comorbidity, with a follow-up of 15 months were studied. 48% were polysensitized, 17% had at least one AIT before the current treatment for another allergen. About half of the products were native allergens, the other half allergoids; subcutaneous applications were preferred in Germany and Spain, the sublingual route in France (mainly drops). About half of the patients received conventional up-dosing schemes, the others either cluster- or rush-schemes. 2.1% of the patients experienced at least one systemic reaction, 88% of the SARs were observed in SCIT (>57.000 applications) and 11% in SLIT (>259.000 applications). Acute urticaria (26 cases) and dyspnoea (17 cases) were only seen in SCIT, AR symptoms in 21 SCIT and 2 SLIT patients. 76% of the SARs occurred during the up-dosing of SCIT. Only 4 SARs were considered severe (0.07% of patients or 0.00007% of injections).

A higher risk of systemic reactions in SCIT was associated with natural allergens vs. allergoids, pollen and animal dander vs. mites, in patients with systemic reactions in the history, and when cluster schemes were applied. Asthma tended to also increase the risk, but remained insignificant. The favourable adverse event records for SLIT is confirmed by a pooled analysis of observations with a 5-grass tablet, showing no SAR; the most frequent treatment-emergent AEs were local-site oropharyngeal reactions consistent with the sublingual route of administration.[60]

A 10-year prospective study on the safety of AIT, which was initiated after a cluster of severe reactions (grade 3 to 5 reactions according to European and WAO classifications) and the introduction of a slower up-dosing protocol in Denmark in 2003, monitored more than 102.000 injections and found a rate of 4 severe reactions per 100.000 injections. [61] According to these authors, the rate of severe reactions should not be higher than 0.004% of the injections. Safety requirements for performing procedures in allergy offices including optimal safety measures (supervision, availability of safety equipment, access to specialized emergency services, etc.) has been recommended. [62]

Mechanisms of Allergen Immunotherapy

The role of mast cells and basophils in allergen immunotherapy

Mast cells and basophils have two types of effect on mechanisms of action of AIT, which can be classified as very early desensitization effects as well as late responses in tissues. Very early events are mainly based on changes in thresholds of rapid degranulation of mast cells and basophils and late effects are based on decreased tissue infiltration and decrease in their mediators. Both early and late effects have been shown to change during AIT and several key papers have been published in the area. Decreased activation of mast cells and basophils may happen within a few hours in ultra-rush venom IT, however it takes 3 to 4 months in OIT. [63, 64] So far there were several mechanisms proposed such as the role of histamine receptor 2 and IgG binding to FcγRIIb. [65-68]

Several studies have been published during the last years to help us better understand the role of basophils in AIT. In a recent study, Glashan and Hamilton have shown that CD32 effect on FcεRI activity inhibition in human basophil. The function of CD32 to inhibit activation of FcεRI in human basophils suggests that measurements of IgG2 and IgG3 antibodies are warranted in IT studies. IgG2 and IgG3 appear to be the most efficacious in recruiting CD32 into interactions with antigen and antibodies on the human basophil. [69]

One recent study indicates that human basophils may not directly respond and be modulated by DC-SIGN and mannose receptor binding nonoxidized mannan-coupled allergoids, as they do not express DC-SIGN and mannose receptors unlike DCs. In addition, the data suggest if DC-SIGN and mannose receptor binding allergens are not IgE bound, they can not activate human basophils. Accordingly, the expression pattern of DC-SIGN and mannose receptors on effector cells contributes to the diversity of allergic and tolerogenic responses. [70]

Biomarkers related to mechanisms of allergen immunotherapy and personalized medicine

AIT represents the most widely used and historically the earliest and most efficient personalized medicine approach. Personalized/precision medicine approaches and novel biomarkers have been extensively investigated during the past years. [71-73] Recent studies found several biomarkers in basophils and mast cells that can be used to determine the efficacy of IT response. Basophil diamine oxidase molecule was proposed in one of the recent studies. In the SCIT, SLIT, and SLIT-TOL groups, percentages of allergen-stimulated diamine oxidase^{+}CRTH2^{+} basophils were found to be greater compared to those in patients in seasonal AR. Similarly, there were lower proportions of CRTH2^{+} basophils expressing surface CD203c^{bright}, CD63, and CD107a. Histamine release from basophil and the serum inhibitory activity for IgE-FAB in all IT groups were found to be significantly higher compared with the seasonal AR group. [74] Abundance and activity of effector cells also change in response to peanut IT. In the long-term follow-up of a randomized multicenter trial using SLIT for peanut allergy, 2-year responders to AIT had significantly lower percentages of CD63^{+} basophils than non-responders. [75]

In a recent study, a fish allergy mouse model mimicking IgE epitope recognition and symptoms of the human disease was developed. Mice and rabbits were immunized with a hypoallergenic Cyp c 1 mutant inhibited IgE binding to Cyp c 1. It is concluded that the blocking IgG antibodies generated against hypoallergenic Cyp c 1 may protect against fish allergy.[76]

In another study on peanut-allergy, basophils and mast cells sensitized with plasma from patients with peanut-allergic but not peanut-sensitized patients showed dose-dependent activation in response to peanut. Depletion of IgG4 from plasma of children with peanut sensitized (and peanut OIT) sensitized to Ara h 1 to Ara h 3 partially restored peanut-induced mast cell activation.[77]

Analysis by using quantitative proteomics of pretreatment sera from patients with grass pollen allergy reveals a strong decrease in rhinoconjunctivitis symptoms after sublingual IT

was observed in patients that have high levels of O-glycosylated sialylated Fetuin-A isoforms. On top of this, a significant upregulation of airway hyperresponsiveness, lung resistance and T_H2 responses after allergic sensitization to ovalbumin was observed after *in vivo* silencing of the *FETUA* gene in BALB/c mice.[78]

New findings on the role of dendritic cells in allergen immunotherapy

Documenting the changes occurring in DCs during AIT is of importance, since the efficiency of allergen-specific T cell responses in IT is directly correlating with the capability of AIT for skewing DCs. A study observed that 5 molecular markers predominantly expressed by blood DCs (ie, C1Q and CD141) or shared with lymphoid cells (ie, FcγRIIIA, GATA3, and RIPK4) reflects changes to regulatory/proallergic responses in peripheral blood. These markers can be used to monitor the early inception of AIT efficacy as early as 2 months after start of AIT. [79]

Induction of Treg cells by tolerizing DC subsets is an important aspect of immune tolerance induction during AIT. Accordingly, it was observed that, IL27 secreted from mature retinoic acid–skewed DCs (DC-RA) was important for inducing $CD25^+$ lymphocyte activation gene 3^+ ($LAG3^+$), $CD49b^-$, $Foxp3^-$ Treg cells *in vitro*. Moreover, β subunit of $IL27^{-/-}$ ($Ebi^{-/-}$) DC-RAs were ineffective in inducing tolerance to food allergens. It is suggested in this study that induction of $Foxp3^-$ Treg cells through regulatory DC inducing IT or usage of regulatory DCs themselves might be a useful strategy for tolerance against food allergies **(Figure 1)** .[80] Additionally, a recent study has identified oral $CD103^-CD11b^+$ classical DCs (cDC) that present sublingual antigen and induce $Foxp3^+$ Treg cells in draining lymph nodes. These results suggest that oral $CD103^-CD11b^+$ cDCs transport sublingual antigens to draining submandibular lymph node and induce antigen-specific $Foxp3^+$ Treg cells, viable strategy for developing cDC-based therapeutic approaches in SLIT.[81]

Role of T cells in allergen immunotherapy

Suppression of effector T cells and induction of Treg cells have been one of the hallmarks of mechanisms of AIT **(Figure 2)**.[82] T cell receptor sequencing of allergen-specific T cell clones during and after IT will mark the new developments for the characterization of T cells in AIT for near future.

A study based on the next-generation sequencing of peanut-proliferative TCRβ in subjects undergoing OIT have found that the peanut allergen-induced proliferation assay leads to a polyclonal response that is extremely diverse., while consistent clones make up only a small fraction of the T cell response. Peanut OIT changes the distribution of this consistent fraction

of allergen-specific T cells, supporting the T cell ‘‘replacement’’ hypothesis as a mechanism of food OIT.[83] In a cohort of peanut-allergic participants, it was observed that allergen-specific $CD4^+$ T cells expand and shift towards an "anergic" T_H2 T-cell phenotype with successful IT, a phenomenon that is not present in both pretreatment participants and healthy controls. [84]

Some recent studies have focused on the immunoregulatory effects of SCIT and intradermal IT and their effects on the generation of Treg cells. Production of OVA-specific IgE was decreased, but IgG2a was increased after epicutaneous immunization with OVA and CpG. Moreover, IL4, IL5, IL10 and IL13 responses and peroxidase activity of eosinophils are suppressed. Reduction of IgE synthesis is dependent on $TCR\alpha\beta^+CD4^+CD25^-$ cells and increase in IgG2a production is tied to the frequency of both $TCR\alpha\beta^+$ and $TCR\gamma\delta^+$ T cells. Further experiments showed that the observed effects are MyD88, IFNγ, and IL17A dependent.[85] Another study on food allergy showed that epicutaneous application of antigen generated a population of gastrointestinal homing LAP^+Foxp3^- Tregs. Mast cells were suppressed by Treg cells with a TGFβ-dependent pathway in the absence of modulatıon for T and B cell responses. This data highlights the immune communication between skin and gastrointestinal tract, and identifies novel mechanisms by which epicutaneous tolerance can suppress food-induced anaphylaxis.[86]

A study investigating the efficacy of intradermal AIT was not clinically effective and resulted in worsening of respiratory allergic symptoms. However, the study was able to show suppressed late-phase skin responses. Intradermal IT increased serum *phleum pratense*–specific IgE levels compared with those in the control arm. T cells expanded from arm biopsies of subjects undergoing intradermal IT had higher T_H2 surface marker CRTH2 expression and lower expression of the T_H1 marker CXCR3, respectively. Interestingly, skin late-phase responses remained inhibited 7 months after treatment. [24]

Innate lymphoid cells and immune regulation

Innate lymphoid cells are arising as the new players that induce allergic inflammation in addition to T_H2 cells.[87] They are in close interaction and cross-talk with T_H2 cells for their T_H2 cytokine and IgE production.[88] ILC2s are dominant in allergic individuals in the circulation and affected tissues may play a role in continuation of the allergic status and resistance to AIT.[89] IL33 stimulated ILC2s block the generation of allergen-specific Treg cells and favor food allergy through production of IL4 in mice. These findings suggest that

blocking IL33/IL33 receptor pathway or suppressing general ILC2 activation represents an innovative approach for the treatment of food allergy.[90]

Circulation and presence of ILC2s in the peripheral blood system and affected tissues of allergic individuals might have a role in the continuation of the allergic status and resistance to AIT.[89]

Recent reports on B cell regulation in allergen immunotherapy

During the last few years, it appeared that B cell regulation is equally important as T cell regulation in AIT. Human Breg cells and particularly their IL10 production were demonstrated to increase during AIT. [91, 92] Peanut OIT induces an early and transient expansion of circulating Ara h 2–specific memory B cells that peaks at week 7. Using a novel affinity selection approach to identify antigen-specific B cells, a recent study demonstrated that the early peanut OIT-induced Ara h 2–specific B-cell receptor repertoire is oligoclonal, somatically hypermutated and shares similar clonal groups among unrelated subjects consistent with convergent selection.[93] Supporting this concept, liposomes simultaneously targeting CD22 and the BCR specific for the major peanut allergen, Ara h 2, can be used to prevent sensitization to Ara h 2. Based on previous studies using other antigens, it can be hypothesized that simultaneous engagement of CD22 and the Ara h 2-specific BCR leads to deletion of the Ara h 2 specific B cells. These findings provide the foundation for the development of a novel therapy for peanut allergy using a highly targeted, antigen-specific approach.[94] On top of these, most peanut allergen-specific B cells express mutated and class-switched antibodies. After IT, multiple B cell clones that recognize narrow allergen epitopes increase in frequency. OIT was also shown to increase the somatic mutation rate of allergen-specific IgG_4. [95] However, in another study, peanut specific binding patterns were very similar for IgE and IgG4 This may indicate that the IgE and IgG4 antibodies are clonally related.[96] Usage of viral vectors might also be a viable strategy for inducing suppressive responses from B cells. A recent study revealed that treatment with innocuous parainfluenza virus vector rhPIV2/IL10 by nasal approach improves pollinosis symptoms without affecting the systemic immune response, plasma IgE and T_H2 cytokine levels, and cytokine profiles of distant lymphoid organs. The data suggest that intranasal rhPIV2/IL10 might be used as a novel therapeutic tool for nasal allergy. [97]

Recent studies have added new biomarkers for detection and targeting Breg cells for controlling allergic inflammation. Selb et al shown that casein-induced food allergic responses in mice can be controlled by IL10-producing $CD5^+$ Breg cells. The population of

IL10-producing $CD5^+$ B cells showed an increase in mesenteric lymph nodes (MLN), but not in spleen or peritoneal cavity in OT mice. Casein induced allergic responses were suppressed by the adoptive transfer of $CD5^+$ B cells from MLN in an allergen-specific and IL10-dependent manner. $CD5^+$ B cells from the spleen and peritoneal cavity did not induce the same effect. This inhibitory effect was shown to be dependent on $Foxp3^+$ Treg cells. Through $Foxp3^+$ Treg cells the mesenteric IL10-producing Breg cells control food allergy, and mesenteric Breg cells can potentially act as a therapeutic regulator for food allergy. [98]

Furthermore, CD23 surface density on B cells of allergic patients correlates with allergen-specific IgE levels and determines allergen uptake and subsequent activation of T cells. It may open new possibilities for controlling T cell-mediated allergic inflammation by targeting this pathway.[99]

Suppressive and tolerogenic B cell frequencies are higher after bee venom IT and in occupations with regular allergen exposure, such as beekeepers. In the study from Boonpiyathad et al. allergic patients and beekeepers show increased frequencies of plasmablasts, PLA-specific memory B cells, and IL10-secreting $CD73^-CD25^+CD71^+$ Br1 cells. After exposure to bee venom, PLA-specific IgG4 switched memory B cells were observed to expand. PLA-specific B cells also showed increased expression of CCR5 after high-dose allergen exposure, while CXCR4, CXCR5, CCR6, CCR7 expression was unaffected.[100]

New findings on regulation of allergen-specific IgE and IgG_4 antibodies and blocking antibody effect of IgG4

Efficacy of sublingual tablets in OIT has been investigated by several studies. Adults with HDM-induced AR with or without conjunctivitis and asthma received 12 developmental units (DU) of HDM sublingual IT tablet MK-8237 (Merck/ALK-Abello), 6 DU of MK-8237, or placebo daily for 24 weeks. MK-8237 with a dose of 12DU, reduced nasal and ocular symptoms. Specific IgE and IgG4 levels increased with the 12 DU and 6 DU treatments versus placebo at week 8, and significant increments with IgE and IgG4 levels were observed at week 24.[12]

In another study, adults with HDM-associated AR were given a daily placebo tablet or sublingual IT tablet STG320 for 6 months. At study entry, serum levels of Der p- and Der f-specific IgE and IgG4 were similar across treatment groups. Within the first 2 treatment months, mite allergen-specific serum IgE increased 5- to 7-fold in the active treatment group, and then gradually decreased, while remained unchanged in the placebo group. Mite allergen-

494 specific serum IgG4 increased over the treatment period in the active treatment group, and
495 showed a small change in the placebo group.[13]

496 Two studies that monitored IgG4 responses during birch pollen IT have reported that, Bet v
497 1– specific IgG4 repertoires induced by birch pollen extract AIT do not broadly expand in the
498 course of treatment in most of the patients. IgG4 diversity increased in 20% of patients with
499 prolonged therapy. [101] Second study applied a chimera-based approach to monitor
500 development of the Bet v 1–specific IgE, IgG1, and IgG4 repertoires during 3 years of AIT in
501 subjects. In most patients treated with allergen chimera peptides Bet v 1–specific IgE levels
502 increased during the early phase of treatment, followed by a gradual decrease. All patients had
503 Bet v 1–specific IgG4, and 64% of patients had Bet v 1–specific IgG1. In these subjects Bet v
504 1–specific IgG4 levels increased later than IgG1 levels. The data suggest that allergen-specific
505 IgE and AIT induced IgG4 and IgG1 repertoires differ among each other, but did not expand
506 over time. Both IgG1 and IgG4 induced during AIT displayed more restricted epitope
507 diversities than IgE antibodies. [102] Furthermore, Bet v 1 derived molecules containing
508 contiguous overlapping peptides were shown to lead to significant increase in serum Bet v 1-
509 specific IgG4 in all but a few patients. Specific IgG4 was significantly increased compared to
510 placebo in both treated groups after treatment as well as during and after pollen season.[40]

511 Studies monitoring efficacy of egg OIT concluded sustained unresponsiveness (a clinical
512 remission with immunological effects) of the immune system to the allergen persists after
513 cessation of long-term egg OIT. Children with egg allergy) received egg OIT for up to 4 years
514 or placebo for 1 year or less. Over time, egg-specific IgG4 levels were significantly greater in
515 sustained unresponsiveness group. Similarly, scores of egg-specific skin prick test (SPT) were
516 observed to be significantly lower in those achieving sustained unresponsiveness. For egg IgE
517 levels and basophil activation, there was a decrease over time in those achieving SU;
518 however, the differences were not significant.[103] For introduction of egg early in life, a recent
519 study found that, IgG4 response to egg proteins and IgG4/IgE ratios were found to be higher
520 in children introduced to egg protein at 12 months. Introduction of whole egg powder into the
521 diets of high-risk infants reduced sensitization to egg white and induced egg-specific IgG4.
522 However, 8.5% of infants exposed to egg protein were not susceptible to this primary
523 prevention.[104] In addition, increased levels of egg white specific IgA and IgA2 were found to
524 be among biomarkers for clinical response to egg OIT.[105]

525 In another study, food allergy-prone IL4raF709 mice treated with specific IgG in the
526 beginning of feeding prevented the development of IgE antibodies, T_H2 responses, and

anaphylaxis upon challenge. When given as supplement to oral desensitization in mice with established IgE-mediated hypersensitivity, IgG antibodies facilitated the re-establishment of tolerance, probably due to favoring the expansion of $Foxp3^+$ Treg along with suppression of existing IgE and T_H2 responses. Adaptive allergic responses were suppressed with IgG and FcγRIIb through their effect on mast cell function. [106]

Predominant Api m 10 sensitization was shown as a risk factor for treatment failure in honey bee venom (HBV) IT. In HBV-allergic patients who had undergone controlled honey bee sting challenge after at least 6 months of bee venom IT, no differences were observed between responders and non-responders regarding levels of IgE sensitization to Api m 1, 2, 3 and 5In contrast, Api m 10 specific IgE was moderately but significantly increased in non-responders. Specific IgG4 induction to Api m 10 was only observed in patients unresponsive to bee venom IT. Results from allergic patients suggest that predominant IgE sensitization to Api m 10, which might be a risk factor for treatment failure in HBV IT.[107]

Several recent studies were directed towards investigating changes in allergen-specific IgE and IgG4 responses during peanut IT and how different methods of IT affect IgE and IgG4 levels during and after peanut IT. Peanut-specific IgE levels are significantly less in children treated with early OIT, and those children are 19 times more likely to be able to consume peanut compared to matched controls. Allergic side effects during early OIT were common, but all were mild to moderate.[52] Uotila et al reported that specific IgE to Ara h 2 and 6, decreased significantly during OIT. Although baseline measurements showed low amounts of specific IgG4 to Ara h 1, 2, 3, 6, 8, 9, and whole peanut extract, specific IgG4 to these allergens significantly increased during OIT. OIT resulted in increased IgG4/IgE ratios for Ara h 1, 2, 3, 6, and whole peanut extract while those for Ara h 8 and 9 remained stable. The strongest correlations were observed for Ara h 2 , 3, 6 and whole peanut extract. The study concluded that serological response to peanut allergens during OIT is directed towards 2S albumins and peanut storage proteins.[108] Burk et al also reported that in patients undergoing peanut SLIT, lower levels of Ara h 2- and Ara h 3-specific IgE, as well as peanut, are among the markers of successful desensitization.[109] Another study confirmed these findings by showing increases in peanut-specific IgG4 levels and IgG4/IgE ratios in peanut EPIT-treated participants, along with trends toward reduced basophil activation and peanut-specific T_H2 cytokines. [51]

The epidemiological study on the genetics and environment of asthma, bronchial hyperresponsiveness and atopy (EGEA) study investigated the the effects of route of exposure to allergens by analyzing IgE and IgG response to 47 inhaled and food allergens collected

from 5 French regions. The study showed that the variability of allergen-specific IgE and IgG frequencies depends on dose and route of exposure and overall immunogenicity of the allergen. Allergen contact by oral route might preferentially induce IgG responses. [110] A multi-center birth cohort study tested IgG and IgG4 responses against 91 allergenic molecules reported that the route of allergen exposure and initial status of IgE sensitization was profoundly important to children's repertoire to foodborne and airborne allergens at 2 years of age, whereas the amount of IgG4 isotype was only marginally involved. These IgG responses are more frequent and stronger in children with current IgE sensitization. [111]

Novel vaccine approaches and their mechanisms

Adjuvanicity of certain allergen modifications and novel adjuvants represent an important part of novel vaccine approaches and related innate mechanisms may define efficacy in AIT.[112] New findings for increasing efficacy for allergoid IT show that low dose therapy with a chemically-modified monomeric allergoid of Der p 2 alone was not fully successful, but supplementation of vitamin D3 was associated with limited changes in the immunological parameters in the lung. In contrast, the most prominent decrease in airway eosinophilia, T_H2 cytokines and concomitant increase of T regulatory cells, IL10 in the lung and Der p 2-specific IgG2a in the serum were induced by allergoid vaccine adjuvated with vitamin D3.[113]

Characterization and peptide sequencing of major allergen types and exploiting their structure for recombinant peptide-based AIT might be a viable strategy for increasing efficacy of AIT. A 19-mer peptide sequence in the immunodominant region of Can f 4 was identified as a potential target for the development of peptide-based AIT, together with the observation that productive T_H2-deviated memory T-cell responses to Can f 4 are observed in allergic but not nonallergic subjects.[114] Allergen peptide carrier vaccines are being developed in line with the same concept. A novel grass pollen peptide carrier vaccine, BM32 have been shown to have lower effect on lymphocyte proliferation compared to grass pollen extract, and BM32 also induced significantly lower secretion of proinflammatory cytokines. [115]

Another study with modified Bet v 1 allergen MBC4 used directed epitope rearrangements combined with a knowledge-based structural modification. The new recombinant allergen was unable to bind IgE from allergic patients. Still, properties to activate specific T cells or induce blocking antibodies were conserved. MBC4 was suggested to be a viable vaccine candidate for treatment of birch and Bet v 1-cross-reactive and food allergies simultaneously.[116]

A major development in this regard is the mannan-coated allergoids for usage as allergen vaccines. Human DCs can capture Glutaraldehyde-polymerized allergoids conjugated to

nonoxidized mannan, which displays *in vivo* hypoallergenicity, much more efficiently than native grass pollen *phleum pratense* allergens or mannan-free glutaraldehyde-polymerized allergoids. Uptake of mannan conjugated allergoids depends on mannose receptor and DC-SIGN-mediated internalization. Non-oxidized mannan allergoid skews human DCs to generate functional $Foxp3^+$ Treg cells through programmed death ligand 1(PD-L1). Immunization of mice with non-oxidized mannan induces a shift to nonallergic responses and increases the frequency of splenic $Foxp3^+$ Treg cells **(Figure 3)**. [117, 118]

New studies on conjugated allergoids show efficacy in control of inflammation in various allergic diseases. A recent study shows that numbers of eosinophils in bronchoalveolar lavage fluid and lung, total IgE and production of IL13 from lung mononuclear cells all decreased significantly in the BALB/c mice challenged and treated with TLR7 ligand-allergen conjugates, in comparison to nDer p 2-treated mice, nDer p 2-conjugate stimulated spleen cells showed significant increase of IFNγ and IL10, as well as IgG2a.

Similar effects were elicited by treatment with OVA-conjugate in an OVA-driven BALB/c model. Cytofluorometric analysis demonstrated that the conjugate expanded IFNγ and IL10-producing memory T cells. $IL10^{-/-}$ and $IL12^{-/-}$ mice were used to confirm the role of IL10 and IFNγ in inducing a protective and balanced redirection the T_H2-mediated airway inflammation. [119] In another study, milk-sensitized mice undergone specific EPIT prevented further sensitization to peanut or HDM. Humoral responses, airway hyperresponsiveness, eosinophilic esophageal infiltration and T_H2 cytokine levels were all reduced with EPIT and sustained for more than 2 months. Moreover, the adoptive transfer of Treg cells from mice undergone EPIT completely prevented sensitization to peanut and peanut induced anaphylaxis. Milk EPIT also enhanced methylation of the GATA3 promoter region. [120]

Targeting the complement system might become a new strategy for increasing efficacy of AIT and inhibiting allergic inflammation. Recently, the complement subunit C1q was identified as a marker for monocyte-derived regulatory DCs supporting the differentiation of IL10 secreting $CD4^+$ T cells with a suppressive activity. Furthermore, C1q expression is upregulated in peripheral blood mononuclear cells of allergic patients in the course of successful AIT. Expression or secretion of molecules such as C1q can confer a potent direct anti-inflammatory function to regulatory DCs, independent of their capacity for expanding the pool of Treg cells. [121]

Allergen Immunotherapy combined with biological and probiotics

Studies on combination of biologicals with AIT seems to be more and more pursued last years. A combined therapy comprising a probiotic together with peanut OIT was recently reported. Cotreatment of probiotic *lactobacillus rhamnosus* CGMCC 1.3724 and peanut OIT in children with peanut allergy was associated with reduced peanut skin prick test responses and peanut-specific IgE levels and increased peanut-specific IgG4 levels. This is the first RCT assessing the novel co-administration of a probiotic and peanut OIT and evaluating sustained unresponsiveness in children with peanut allergy. [46]

Similarly, for milk IT, combining omalizumab therapy with milk OIT led to distinct alterations in basophil reactivity but not T cell responses. [122] Following the same concept, a multicenter RCT study has shown that long-term usage of omalizumab has benefits for long-term efficacy. Subjects continuing omalizumab had significantly better asthma control. Discontinuation of omalizumab was associated with an increase in free IgE levels and an increase in basophil expression of the high-affinity IgE receptor. [123] Another study investigating the effects of omalizumab treatment in conjunction with OIT for milk allergy found that casein-specific IgE levels were significantly increased in omalizumab treated group at 4^{th} month and then reduced at 32^{nd} month. In the omalizumab treated group, the percentage of $CD63^{+}$ basophils were found to be lower compared to the placebo group at 28^{th} month. Afterward at 32^{nd} month the percentage of $CD63^{+}$ basophils in the omalizumab treated group reached to a similar level as the placebo group. [53]

Short-term grass pollen SCIT under the umbrella of anti-IL4 was investigated in a recent study. Treatment with anti-IL4 and SCIT compared with SCIT alone induced decreased allergen-specific IL4-producing cell counts. Dual IL4/IL10-producing cells were induced in both active treatment arms during the pollen season. There was no additional benefit of the combination of anti-IL4 with SCIT over SCIT alone. [43]

Conclusion

Recent developments in the clinics and cellular and molecular mechanisms of AIT aim at enhancing clinical and immunological tolerance, decrease side effects and increase efficacy. Several clinical trials have been successfully completed during the last couple of years reaching an endpoint. Hypoallergenic, recombinant allergen and allergoid vaccines, as well as usage of probiotics, vitamins and biologics as supplements to support AIT are expected to enhance efficacy. Many novel developments in molecular mechanisms that affect early desensitization, T and B cell tolerance, specific antibody regulation and induction of IgG4 and several key molecules that may act as biomarkers are continuously developing (**Online**

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660 **Repository Table 2**). As new technologies and novel strategies emerge, we are in need of
661 more research into the mechanisms, biomarker discovery and disease phenotyping for AIT.
662 AIT represents the most common and one of the very few human *in vivo* relevant antigen-
663 specific immune tolerance models, and there will always be take home messages for other
664 immune tolerance-related conditions, such as autoimmunity, organ transplantation, chronic
665 infections, cancer, and recurrent abortions.

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Figure Legends

FIG 1. Cellular mechanisms of allergen-specific immunotherapy. Immune tolerance inducing conditioning and allergen uptake of regulatory dendritic cells induces Treg and B regulatory (Breg) cells. Allergen-specific regulatory cells induce tolerance to allergen in the periphery, and this suppressive environment regulates the effector cells of allergy. IL10 and TGFβ production from Treg cells suppress T_H2 cell proliferation and T_H2 cytokines, while inducing IgG4 and IL10 from allergen specific B cells. Absence of T_H2 cytokines in turn decreases basophil activation, mast cell, eosinophil and ILC2 activity.

FIG 2. Mechanism of effect for the mannan conjugated allergoid vaccines in allergen-specific immunotherapy. Allergen of interest is polymerized and then glycoconjugated with mannan residues for the synthesis of allergoids for vaccine usage. Mannan receptor and DC-SIGN molecule expressed on human dendritic cells facilitates uptake of allergoids and skews dendritic cells to a regulatory phenotype. IL10 secretion, together with presentation of allergoid derived peptides drives allergen specific naïve T cell pool into differentiation as Treg cells. PDL1 on DCs plays an important role in this context (reference 114 Sirvent et al) .

FIG 3. Mechanism of effect for retinoic acid supplementation in allergen-specific immunotherapy. Retinoic acid and LPS pulsed human dentritic cells, together with uptake of allergen become retinoic-acid skewed dendritic cells (DC-RA). These dendritic cells with regulatory phenotype are capable of secreting IL10, TGFβ, as well as IL27. IL10 and TGFβ in turn suppresses proliferation and cytokine secretion from Th2 cells, whereas IL27 drives Th2 and naïve T cells into differentiating as Treg cells, establishing tolerance to allergen in the periphery.

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1113 **Table 1.** Randomized, double-blind, placebo-controlled clinical trials in allergic
1114 rhinitis/rhinoconjunctivitis - Specific routes of administration / allergen preparations /
1115 combination of treatments
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		ALLERGIC RHINITIS/RHINOCONJUNCTIVITIS - Specific routes of administration / allergen preparations / combination of treatments Randomized, double-blind, placebo-controlled clinical trials					
		First author, year	**Product**	**Study population**	**route of administration and dosing scheme**	**Study design; Total number of patients randomized**	**Primary outcome; p-value**
INTRADERMAL	**GRASS**	Slovick et al., 2016	Timothy grass pollen extract (*Phleum pratense)*	Adults (18-65 years) with grass-pollen-induced allergic rhinitis	7 pre-seasonal intradermal injections; every 2 weeks; injections containing 10 BU (7 ng of the major allergen Phl p 5) (BU, biological units)	Single-center, randomized, double-blind, placebo-controlled trial; n=93	Daily combined symptom-medication scores during the following grass pollen season, AIT versus placebo; p=0.80 (Remark: worse nasal symptoms and asthma symptoms in the active intradermal treatment group; secondary endpoints)
EPICUTANEOUS	**GRASS**	Senti et al., 2015	Grass pollen extract in petrolatum, 200 IR/ml	Adults (18-65 years) with grass-pollen induced allergic rhinoconjunctivitis	Epicutaneous; six patches, each applied to the upper arm and kept there for 8 h, at weekly intervals, pre-seasonal	Single-center, randomized, double-blind, placebo-controlled trial; n=99	Visual analogue scale to rate general improvement or deterioration on a scale ranging from -100 mm (worst conceivable symptom exacerbation) to +100 mm (total symptom relief); - after treatment year, p=0.003 compared to placebo - after treatment free follow-up year, p=0.430 compared to placebo
ILIT	**GRASS/BIRCH**	Hylander et al., 2016	Birch- or grass-pollen extract	Adults (18-65 years) with grass/birch-pollen-induced allergic rhinoconjunctivitis	Intralymphatic injections; three injections at 3- to 4-week intervals	Single-center, randomized, double-blind, placebo-controlled trial; n=36 (Remark: expansion of previously reported trial: First cohort, n=15 Second cohort, n=21)	Change in pollen season-associated allergic symptoms (at the end of the first allergy season after treatment, patients indicated on a visual analogue scale (VAS) how their most recent seasonal allergic symptoms were in comparison with symptoms experienced during the pollen season prior to treatment); p=0.047 (Remark: results not separately reported for new cohort)
PEPTIDES INTRADERMAL	**GRASS**	Ellis et al., 2017	Grass allergen peptides (short T cell epitope peptides or SPIREs)	Adults (18-65 years) with grass-pollen-induced allergic rhinoconjunctivitis	Pre-seasonal intradermal injections; 3 regimens vs placebo: (a) 6 nmol at 2-week intervals for a total of 8 doses "8x6Q2W" (b) 12 nmol at 4-week intervals for a total of 4 doses "4x12Q4W" (c) 12 nmol at 2-week intervals for a total of 8 doses "8x12Q2W"	Multicenter, randomized, double-blind, placebo-controlled trial; n=282	Change from baseline in total rhinoconjunctivitis symptom score across days 2 to 4 of a 4-day post-treatment challenge in the environmental exposure unit after the grass pollen season; (a) "8x6Q2W" versus placebo, p=0.035 (b) "4x12Q4W" versus placebo, p=0.260 (c) "8x12Q2W" versus placebo, p=0.452

PEPTIDE SCIT	**BIRCH**	Spertini et al., 2016	Bet v 1-derived peptides ('contiguous overlapping peptides', COP)	Adults (18-55 years) with birch-pollen-induced allergic rhinoconjunctivitis	5 pre-seasonal subcutaneous injections; 2 regimens vs placebo: (a) 25 µg on day 1, 50 µg on days 8, 15, 29, and 57 (b) 50 µg on day 1, 100 µg on days 8, 15, 29, and 57	Multicenter, randomized, double-blind, placebo-controlled trial; n=240	"Combined daily rhinoconjunctivitis symptom and medication score" (1:1 combination of mean daily symptom scores and rhinoconjunctivitis medication scores); (a) 50 µg group vs placebo, p=0.015 (b) 100 µg group vs placebo, p=0.180
PEPTIDE SCIT		Zieglmayer et al. 2016	Grass pollen B cell epitope-based peptide vaccine BM32 (four recombinant fusion proteins consisting of non-allergenic peptides derived from the IgE-binding sites of major grass pollen allergens fused to hepatitis B virus-derived PreS)	Adults (18-60 years) with grass-pollen-induced allergic rhinitis	Subcutaneous injections; three injections with approximately one month interval; 3 regimens vs placebo: (a) 10 ug BM32 (b) 20 ug BM32 (c) 40 ug BM32	Single-center, randomized, double-blind, placebo-controlled trial; n=71	The difference in total nasal symptom score (TNSS) before and after treatment; - effect compared to placebo: (a) 10 ug BM32, p-value not given (b) 20 ug BM32, p-value not given (c) 40 ug BM32, p-value not given - effect compared to baseline: (a) 10 ug BM32, p=0.102 (-10%) (b) 20 ug BM32, p=0.030 (-24%) (c) 40 ug BM32, p=0.003 (-20%) placebo, p=0.084 (-17%)
COMBINED SCIT + anti-IL-4	**GRASS**	Chaker et al., 2016	Grass pollen extract (Alutard Avanz Phleum) + Human therapeutic antibody to human IL-4 (VAK694)	Adults (18-60 years) with grass-pollen-induced allergic rhinitis	Pre-seasonal subcutaneous AIT; 13 weeks; conventional weekly updosing schedule to a suboptimal dose of 30,000 SQ + Intravenous anti-IL4 (3 mg/kg) every 4 weeks (overall 4 doses) 3 groups: (a) grass SCIT + anti-IL4 i.v. (b) grass SCIT + placebo i.v. (c) placebo SCIT + placebo i.v.	Single-center, randomized, double-blind, placebo-controlled trial; n=37	Induction of sustained tolerance to allergen 12 months after the end of treatment, assessed by cutaneous late-phase response to allergen; Grass SCIT + anti-IL4 vs placebo: $p<0.05$ Grass SCIT vs placebo: $p<0.01$ Grass SCIT + anti-IL4 vs Grass SCIT alone: NS (+ no significant difference between the groups in effect on symptoms of AR measured with VAS)

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1120 **Table 2.** Recent developments in Immunotherapy of food allergy

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		FOOD ALLERGY					
		First author, year	**Product, manufacturer**	**Study population**	**Treatment time, route of administration, dosing scheme**	**Study design, Total number of patients**	**Primary outcome, p-value**
PEANUTT	**EPICUTANEOUS**	Jones et al., 2017	Viaskin Peanut patch (epicutaneous delivery system containing a dry deposit of a formulation of peanut protein extract)	Children and young adults (4-25 years) with peanut allergy	Epicutaneous patch delivery system; 52 weeks; week 1, 3 h/d - week 2, 6 h/d - week 3, 12 h/d - followed by patch application for 24 h/d; 2 regimens vs placebo: (a) Viaskin Peanut 100 µg (b) Viaskin Peanut 250 µg	Multicenter, randomized, double-blind, placebo-controlled trial; n=74	"Success after 52 weeks" defined as passing a 5044-mg protein oral food challenge or achieving a 10-fold or greater increase in successfully consumed dose from baseline to week 52 (a) Viaskin Peanut 100 µg vs placebo, p=0.005 (b) Viaskin Peanut 250 µg vs placebo, p=0.003 (45.8% in 100 µg group, 48.0% in 250 µg group and 12.0% in placebo group)
		Sampson et al., 2015 (abstract)	Viaskin Peanut patch (epicutaneous delivery system containing a dry deposit of a formulation of peanut protein extract)	Children - adults (6-55 years)	Epicutaneous patch delivery system; 52 weeks; 3 regimens vs placebo: (a) Viaskin Peanut 50 µg (b) Viaskin Peanut 100 µg (c) Viaskin Peanut 250 µg	Multicenter, randomized, double-blind, placebo-controlled trial; n=221	Achieving a post-treatment peanut protein eliciting dose ≥1000 mg or achieving a peanut protein eliciting dose 10-fold greater than at entry; (a) 50 µg vs placebo, p-value not given (b) 100 µg vs placebo, p-value not given (c) 250 µg vs placebo, p=0.011

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	EARLY OIT	Vickery et al., 2017	Peanut protein OIT with 12% lightly roasted, partially defatted peanut flour	Young children (9 - 36 months) with peanut allergy	Early oral IT; 12 to 36 months; initial-day dose escalation, followed by build-up phase and maintenance phase; target maintenance doses of: (a) 300 mg/d (300 mg peanut flour plus 2700 mg of placebo filler) (b) 3000 mg/d	Single-center, randomized, double-blind, trial; n=37 No blinded placebo control group, but 154 matched standard-care controls	Proportion of subjects achieving sustained unresponsiveness (SU) at 4 weeks after discontinuing early intervention OIT (4-SU); (a) 300 mg/d arm: 85%; (b) 3000 mg/d arm: 71% over a median of 29 months (p=0.43 for difference between both arms) (+ 20 out of 154 matched standard-care controls were deemed OFC-eligible over an average follow-up of 3.6 years: 6 of them passed the OFC; no spontaneous peanut tolerance was observed in the other control patients → overall proportion in control group: 4%)
	OIT + Anti-IgE	MacGinnitie et al., 2017	Peanut OIT with peanut flour (50%) + Xolair (Omalizumab)	Children and young adults (7-25 years) with peanut allergy	12 weeks treatment with omalizumab/placebo, followed by combined treatment with omalizumab/placebo and peanut updosing oral IT, followed by discontinuation of omalizumab (or placebo) and ongoing maintenance OIT	Single-center, randomized, double-blind, placebo-controlled trial; n=37	The ability to tolerate 2000 mg of peanut protein 6 weeks after withdrawal of omalizumab; p<0.01 (79% in omalizumab group vs 12% in placebo group)

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	OIT + Probiotics	Tang et al., 2015	- Peanut OIT with peanut flour (50%) - Bacterial adjuvant: *Lactobacillus rhamnosus*	Children (1-10 years) with peanut allergy	Oral administration; daily for 18 months; initial-day dose escalation, followed by 2-weekly updosing (8 months), followed by maintenance phase (10 months);	Single-center, randomized, double-blind, placebo-controlled trial; n=62	Sustained unresponsiveness (SU), defined as passing both the oral food challenge at the end of OIT (T1) and after 2 or more weeks of discontinuation of OIT and peanut elimination; Active group (active OIT + active probiotic) vs placebo group (placebo OIT + placebo): p<0.001 (82.1% in active group vs 3.6% in placebo group)
	SLIT vs OIT	Narisety et al., 2015	Peanut extract for sublingual administration and peanut powder for oral administration	Children and adolescents (6 -21 years) with peanut allergy	Oral or sublingual administration; daily; initial dose-escalation on first day, followed by 1/2-weekly updosing, followed by 12 months maintenance	Single-center, RCT; n=21	Induction of peanut desensitization, which was defined as a 10-fold increase in the oral food challenge (OFC) threshold after 12 months of therapy; OIT vs SLIT, p=0.76 (64% vs 70%) Remark: Increase in median challenge dose after 12 months of therapy: OIT vs SLIT, p=0.01 (141-fold vs 22-fold)
EGGG	**HYDROLYSED**	Giavi et al., 2016	Low allergenic hydrolyzed egg (HydE) preparation	Children (1-5.5 years) with IgE-mediated egg allergy	Oral administration; daily for 6 months	Single-center, RCT; n=29	Result of an open oral food challenge at the end of the treatment, compared to placebo; p=0.66 (36% in active group vs 21% in placebo group)

	OIT	Caminiti et al., 2015	Dehydrated egg white	Children (4-11 years)	Oral administration; 4 months; weekly administration, doubling the dose every week until week 16 to achieve a cumulative dose of 4 g	Two-center, randomized, double-blind, placebo-controlled trial; n=31	Achievement of desensitization after the 4-month randomized period of OIT with dehydrated egg white; p<0.001 (94% in active group vs 0% in placebo group) (Remark: sustained unresponsiveness (SU) after 3 months of egg avoidance was achieved in only 31% of the active group)
COW'S MILKK	**OIT + anti-IgE**	Wood et al., 2016	Nonfat dry powdered milk + Xolair (omalizumab)	Children, adults (7 - 35 years) with IgE-mediated cow's milk allergy	Initial 16 months treatment with omalizumab/placebo (blinded) injections every 2 or 4 weeks; open-label milk OIT started 2 weeks after month 4 of omalizumab/placebo; additional 12 months of omalizumab in active group (unblinded) and milk OIT in both groups; ± additional 8 weeks of milk OIT alone Milk OIT: initial-day dose escalation, followed by 2-weekly build-up phase and maintenance phase	Multicenter, randomized, "double-blind", placebo-controlled trial ; n=57	Development of sustained unresponsiveness (SU) at month 28 (i.e. end of treatment milk OIT + omalizumab) and at month 32 (i.e. after 2 months off of milk OIT) At month 28, p=0.18 (88.9% in omalizumab group versus 71.4% in placebo group) At month 32, p=0.42 (48.1% in omalizumab group versus 35.7% in placebo group) (remark: significantly reduced treatment-related adverse reactions in omalizumab group)

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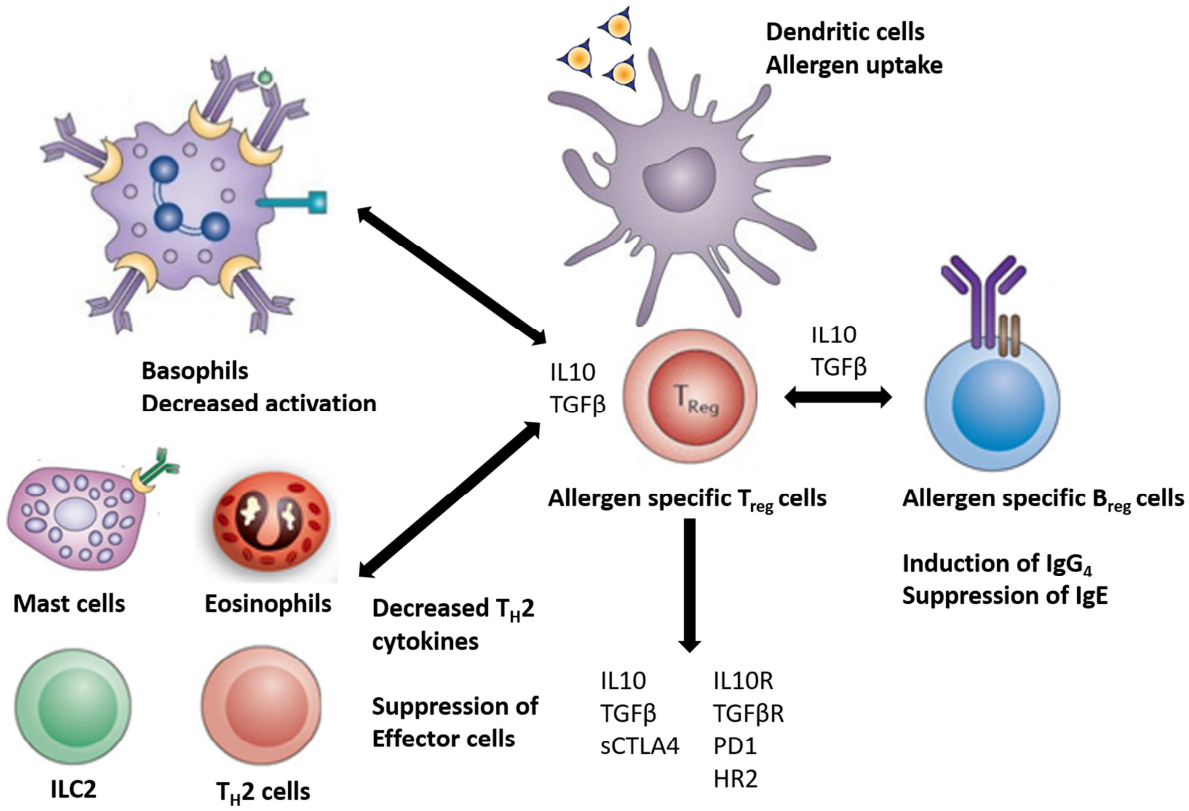

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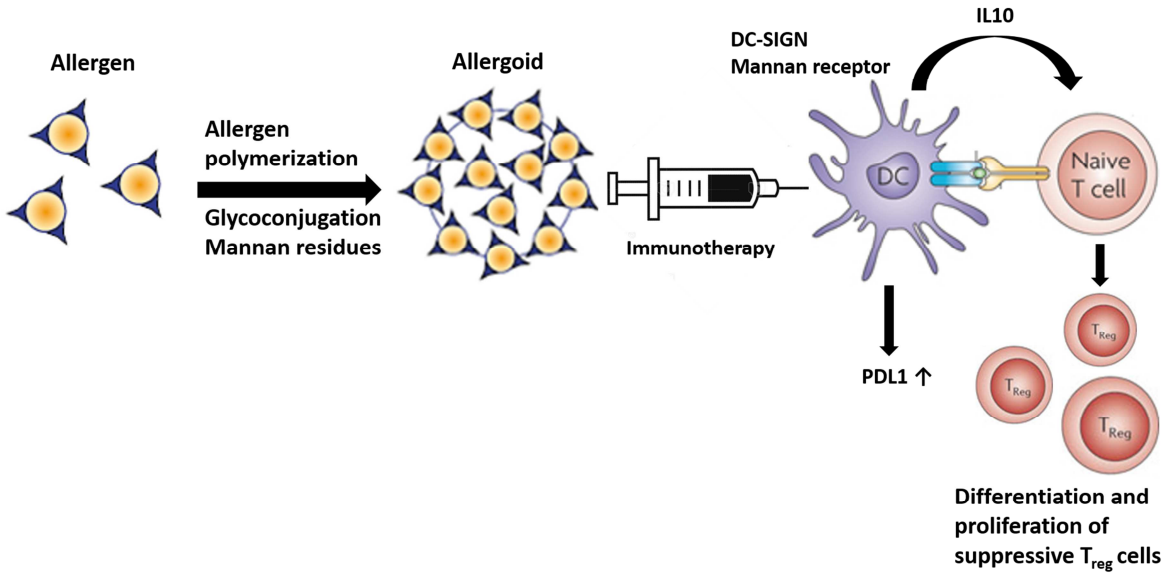

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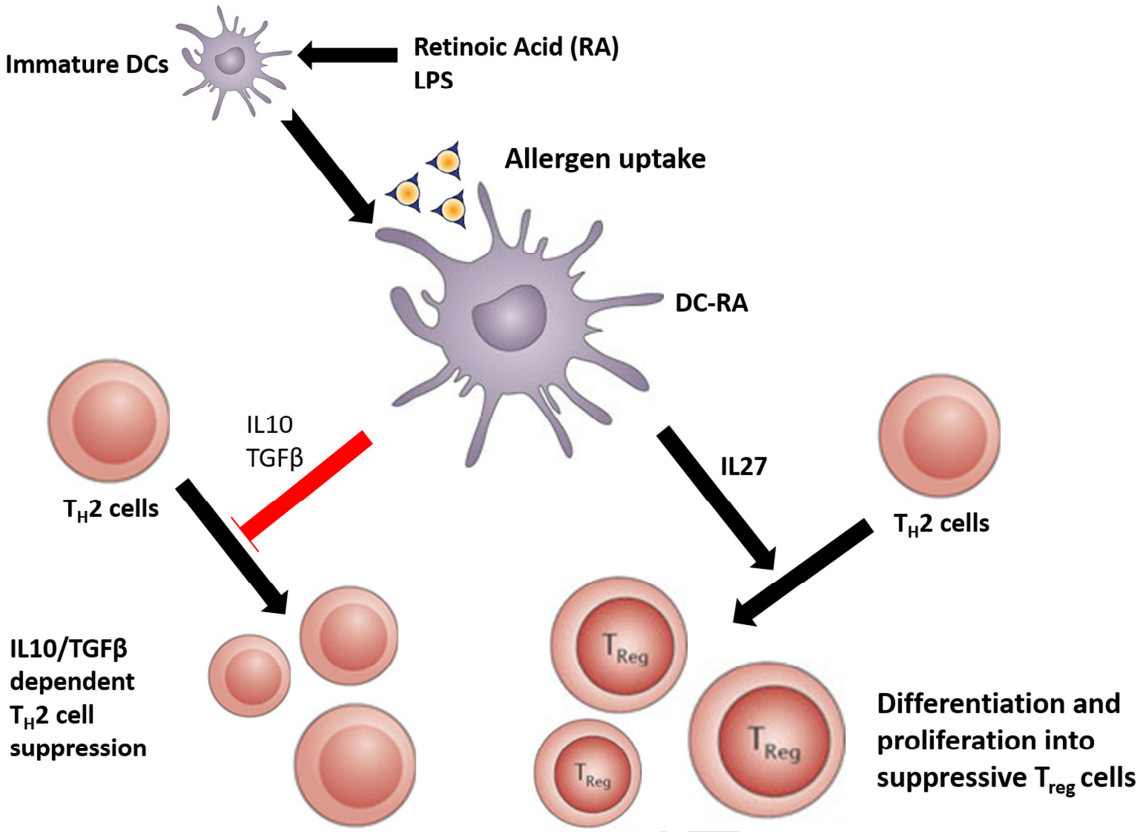

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Advances and Highlights in Allergen Immunotherapy: On the way to sustained clinical and immunologic tolerance

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Online Repository Table 1. Randomized, double-blind, placebo-controlled clinical trials in ALLERGIC RHINITIS/RHINOCONJUNCTIVITIS/ASTHMA – SLIT or SCIT for house dust mite allergy

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			ALLERGIC RHINITIS/RHINOCONJUNCTIVITIS/ASTHMA – SLIT or SCIT for HDM Randomized, double-blind, placebo-controlled clinical trials					
			First author, year	**Product**	**Study population**	**route of administration and dosing scheme**	**Study design; Total number of patients randomized**	**Primary outcome; p-value**
SLIT	**HDM**	**AR/C**	Nolte et al., 2015	HDM SLIT-tablet (D.*pteronyssinus* and D.*farinae*)	Adults (≥ 18 years) with HDM-induced allergic rhinitis (with or without conjunctivitis and asthma)	Sublingual tablets; daily for 24 weeks; 2 regimens vs placebo: (a) 6 SQ-HDM (b) 12 SQ-HDM (SQ: standardized quality)	Single-center, randomized, double-blind, placebo-controlled trial; n=124	Total nasal symptom score (TNSS) during chamber challenge at week 24; (a) 6 SQ-HDM vs placebo, p=0.003 (b) 12 SQ-HDM vs placebo, p<0.001
			Demoly et al., 2016	HDM SLIT tablet (D.*pteronyssinus* and D.*farinae*)	Adults (18-65 years) with HDM-induced allergic rhinitis (with or without conjunctivitis and asthma)	Sublingual tablets; daily 1 year; 2 regimens vs placebo: (a) 6 SQ-HDM (b) 12 SQ-HDM (SQ: standardized quality)	Multicenter, randomized, double-blind, placebo-controlled trial; n=992	Total combined rhinitis score (TCRS: the sum of rhinitis symptom and medication scores) during the efficacy assessment period (± last 8 treatment weeks); (a) 6 SQ-HDM vs placebo, p=0.002 (b) 12 SQ-HDM vs placebo, p=0.001 (Remark: 6 SQ-HDM comparable with 12 SQ-HDM, however, results were in general more robust for the 12 SQ-HDM dose)
			Nolte et al., 2016	HDM SLIT tablet (D.*pteronyssinus* and D.*farinae*)	Adolescents and adults (≥ 12 years) with HDM-induced allergic rhinitis (with or without conjunctivitis and asthma)	Sublingual tablets; daily for approximately 52 weeks; 12 SQ-HDM dose	Multicenter, randomized, double-blind, placebo-controlled trial; n=1482	Total combined rhinitis score (TCRS) during the efficacy assessment period (± last 8 treatment weeks); AIT versus placebo; p<0.001
			Okubo et al., 2016	HDM SLIT tablet (D.*pteronyssinus* and D.*farinae*)	Adolescents and adults (12-64 years) with HDM-induced allergic rhinitis	Sublingual tablets; daily for approximately 12 months; 2 regimens vs placebo: (a) 6 SQ-HDM (10,000 JAU) (b) 12 SQ-HDM (10,000 JAU) (JAU= Japanese Allergy Units) (initial dose-escalation)	Multicenter, randomized, double-blind, placebo-controlled trial; n=946	Total combined rhinitis score (TCRS) during the efficacy assessment period (± last 8 treatment weeks); (a) 6 SQ-HDM vs placebo, p<0.001 (b) 12 SQ-HDM vs placebo, p<0.001 (Comparable effect with both doses)

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SCIT	HDM		Roux et al., 2016	HDM SLIT tablet (D.*pteronyssinus* and D.*farinae*)	Adults (18-55 years) with HDM-induced allergic rhinitis	Sublingual tablets; daily for ± 6 months; 3 regimens vs placebo: (a) 100 IR (b) 300 IR (c) 500 IR (IR, index of reactivity)	Multicenter, randomized, double-blind, placebo-controlled trial; n= 355	Change from baseline to the end of treatment in the area under the curve of the Rhinitis Total Symptom Score (RTSS) over the 4 hours of the challenge; (a) 100 IR vs placebo, NS (b) 300 IR vs placebo, NS (c) 500 IR vs placebo, p=0.0427 (Remark: trend for dose dependent effect)
			Okamoto et al., 2017	HDM SLIT tablet (D.*pteronyssinus* and D.*farinae*)	Adolescents and adults (12-64 years) with HDM-induced allergic rhinitis	Sublingual tablets; daily for 52 weeks; 2 regimens vs placebo: (a) 300 IR (b) 500 IR (initial dose-escalation with 100 IR tablets)	Multicenter, randomized, double-blind, placebo-controlled trial; n=968	The average Adjusted Symptom Score (AASS) in the last eight weeks of the 52-week treatment period; (a) 300 IR vs placebo, p<0.001 (b) 500 IR vs placebo, p<0.001 (Slightly better trend with 300 IR than with 500 IR, but no significant difference)
		ASTHMA	Virchow et al., 2016	HDM SLIT tablet (D.*pteronyssinus* and D.*farinae*,)	Adults (17-83 years) with HDM allergy–related asthma not well controlled by ICS or combination products, and with HDM allergy–related rhinitis	Sublingual tablets; daily for a duration of 7 to 12 months; 2 regimens vs placebo: (a) 6 SQ-HDM (b) 12 SQ-HDM	Multicenter, randomized, double-blind, placebo-controlled trial; n=834	"Time to first moderate or severe asthma exacerbation during the ICS reduction period" (assessed during the last 6 months of the trial when ICS was reduced by 50% for 3 months and then completely withdrawn for 3 months) (a) 6 SQ-HDM vs placebo, p=0.045 (b) 12 SQ-HDM vs placebo, p=0.03
		AR/C	Pfaar et al., 2016	HDM allergoid (glutaraldehyde-modified HDM extract; D.*pteronyssinus* and D.*farinae*);	Adults (18-60 years) with HDM-induced allergic rhinoconjunctivitis	Subcutaneous; initial updosing with 6 weekly injections, followed by monthly maintenance injections; ± 12 months; 4 regimens vs placebo: (a) 6667 AUeq/ml (b) 20 000 AUeq/ml (c) 50 000 AUeq/ml (d) 100 000 AUeq/ml	Multicenter, randomized, double-blind, placebo-controlled trial; n=290	The clinical response to Titrated Nasal Provocation Test (TNPT) after 12 months of treatment (Lebel symptom score); (a) 6667 AUeq/ml vs placebo, p=0.140 (b) 20 000 AUeq/ml vs placebo, p=0.040 (c) 50 000 AUeq/ml vs placebo, p=0.022 (d) 100 000 AUeq/ml vs placebo, p=0.049

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Online Repository Table 2. Summary of new findings and biomarkers according to cell types in AIT

	First author, year	Biomarkers	Mechanisms of function
Mast Cell and Basophils	MacGlashan D, Jr., Hamilton RG 2016	CD32	CD32 inhibits activation of FcεRI in human basophils. IgG2 and IgG3 appear to be the most efficacious in recruiting CD32 into interactions
	Das M et al., 2017	DC-SIGN, Mannose receptors	Human basophils were found not express DC-SIGN. The expression pattern of DC-SIGN and mannose receptors on effector cells contributes to the diversity of allergic and tolerogenic responses
	Shamji MH et al., 2015	Diamine oxidase	Percentage of diamine oxidase$^+$ CRTH2$^+$ basophils were higher in patients taking SCIT and SLIT.
Dendritic Cells	Gueguen C et al., 2016	C1Q CD141 FcγRIIIA GATA3 RIPK4	These markers can be used to monitor early inception of AIT efficacy.
	Dawicki W et al., 2017	IL27	IL27 secreted from mature retinoic acid–skewed DCs (DC-RA) was important for inducing CD25$^+$ lymphocyte activation gene 3$^+$ (LAG3$^+$), CD49b$^-$, Foxp3$^-$ Treg cells *in vitro*
T Cells	Majewska-Szczepanik M et al., 2016	TCRαβ$^+$CD4$^+$CD25$^-$ TCRαβ$^+$ and TCRγδ$^+$ T	After epicutaneous immunization, decreased IgE production is found to be related to TCRαβ$^+$CD4$^+$CD25$^-$ cells and increased IgG2a synthesis is tied to the frequency of TCRαβ$^+$ and TCRγδ$^+$ T cells.
Innate Lymphoid Cells	Lombardi V et al., 2016	ILC2 and IL-33	IL33 stimulated ILC2s block the generation of allergen-specific Treg cells which is important for continuation of the allergic status and resistance to AIT.
B cells	Kim AR et al., 2016	IL10-producing CD5$^+$ Breg	Casein-induced food allergic responses in mice can be inhibited by IL10-producing CD5$^+$ Breg cells. This inhibitory effect was shown to be dependent on Foxp3$^+$ Treg cells.
	Boonpiyathad T et al., 2017	IL10-secreting CD73$^-$ CD25$^+$CD71$^+$ Br1	IL10-secreting CD73$^-$CD25$^+$CD71$^+$ Br1 show higher frequency after bee venom IT and regular allergen exposure (e.g. Beekeepers)
		Egg white specific IgA and IgA2	Increased levels of egg white specific IgA and IgA2 were found to be among biomarkers for clinical response to egg OIT
New Biomarker	Caillot N et al., 2016	O-glycosylated sialylated Fetuin-A (*FETUA*)	Airway hyperresponsiveness, lung resistance and T_H2 responses were increased after silencing of *FETUA* gene