Advances and highlights in allergen immunotherapy: On the way to sustained clinical and immunologic tolerance

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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 developed significantly. 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 IgG_4 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 biological agents with AIT is highlighting current advances. Development of allergoids and recombinant and hypoallergenic vaccines to skew the immune response from IgE to IgG_4 and regulation of dendritic cell, mast cell, basophil, innate lymphoid cell, T-cell, and B-cell responses to allergens are also discussed in detail. (J Allergy Clin Immunol 2017;140:1250-67.)

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In this review a number of recently published key developments and publications in the field of allergen immunotherapy (AIT) 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 increase considerably the efficacy of specific AIT products and develop the field into the future of evidence-based treatment.[1] Both subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT) have been established as viable and safe procedures if administered with the correct products.[2] The international consensus consortium on AIT 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 the efficacy and safety of SCIT and SLIT to allow for best practices in AIT treatment 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 AIT: 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 standardized by 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 and spans all AIT vaccines.[3] In addition, state-of-the art studies with AIT products should provide

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Abbreviations used

AIT: Allergen immunotherapy
AR: Allergic rhinitis
Breg: Regulatory B
cDC: Classical dendritic cell
COP: Contiguous overlapping peptide
CRTH2: Chemoattractant receptor–homologous molecule expressed on T_H2 lymphocytes
DC: Dendritic cell
DC-SIGN: Dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin
DU: Developmental units
EPIT: Epicutaneous immunotherapy
Foxp3: Forkhead box protein 3
HBV: Honeybee venom
HDM: House dust mite
ILC2: Type 2 innate lymphoid cell
OIT: Oral immunotherapy
OVA: Ovalbumin
PLA: Phospholipase A
RCT: Randomized controlled trial
SAR: Systemic adverse reaction
SCIT: Subcutaneous immunotherapy
SLIT: Sublingual immunotherapy
TCR: T-cell receptor
Treg: Regulatory T

evidence for efficacy and tolerability. European directives classified allergen products as medicinal products, providing specifications for these 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. Because allergen products are meant to modulate immune tolerance in the patient, such prerequisites seem more than justified to ensure efficacious treatment.

Although a large number of national and international guidelines for AIT have been developed,[2] they do not base their recommendations on established doses per product but rather on use of meta-analyses of registered and nonregistered products, for which dose-finding studies might 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 any other product; there is no "class effect" caused by the heterogeneity in composition and dosing of individual products.[4] This becomes specifically obvious for AIT in children, claims on asthma prevention, and so on, but also excludes claims on mixtures unless optimal dosing is used per allergen in the mixture. However, this meets obvious limitations regarding safety. A recent World Allergy Organization statement indicates the need for product-specific evidence-based AIT.[5]

In the past, we were used to honoring meta-analyses for their high scientific status; however, there is some criticism on flaws in technique and 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 might generate confusion among physicians.[8] Flaws can result from incorrect selection of trials, inappropriate use of evaluation parameters for the analysis, bias toward unpublished studies, unsuitable analyses, and overinterpretation 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-the-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 of treatment and, optimally, a study in adults over 3 years and 2 years of follow-up for claims on long-term efficacy and disease modification.[5,9] One such study tested grass pollen products in patients with moderate-to-severe AR undergoing grass pollen SLIT and SCIT for 2 years with a 3-year follow-up and found no significant improvement of nasal response over placebo control at the third year. However, grass pollen–specific IgE levels were decreased after 2 and 3 years in patients undergoing SCIT.[10]

RECENT ADVANCES IN AIT 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 showed that 65 to 130 million persons worldwide might have 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; thus far, however, there has been a deficit in adequately studied AIT products for patients with HDM allergy. Furthermore, international guidelines restricted AIT application in patients with uncontrolled asthma.[4]

During the past 2 years, dose-finding studies in exposure chambers[12,13] and classical state-of-the-art field trials[14-17] have provided strong evidence for the efficacy, tolerability, and safety of HDM sublingual immunotherapy tablets in both adults and adolescents (>12 years) with AR (see Table E1 in this article's Online Repository at www.jacionline.org).[12-17]

Moreover, for the first time, a large multicenter randomized controlled trial (RCT) has studied the effect of HDM sublingual tablets in adults (n = 834) with HDM allergy–related asthma that was not well controlled by inhaled corticosteroids or combination treatment. The study showed a significantly reduced risk for moderate or severe asthma exacerbations, as defined by American Thoracic Society–European Respiratory Society criteria during a 6-month period of inhaled corticosteroid reduction. Treatment-related adverse events were common but were primarily mild-to-moderate local reactions. Further studies are needed to evaluate the long-term efficacy and safety of HDM AIT in asthmatic patients.[18]

Compared with the accumulating evidence in HDM tablets, there is only 1 recent publication of an RCT evaluating the efficacy of HDM SCIT.[19] This dose-finding study demonstrated significant improvement in the clinical response to a titrated nasal

provocation test after 12 months of treatment with HDM allergoid SCIT in the 3 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 2 higher dosages for the last 8 weeks of study, potentially translating the efficacy observed in the nasal challenge to clinics. However, because of a higher incidence of adverse events in the highest dose group, the study favored 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 been suggested previously that AIT 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 (age, 5-9 months) at high hereditary risk to atopy (≥2 first-degree relatives with allergic disease) with negative skin prick test responses to common food allergens 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 with 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 the International Statistical Classification of Diseases, 10th Edition, caused by AIT has been provided in a "real-life," large, retrospective, cohort study by using routine health care data from German National Health Insurance beneficiaries.[21] The cohort consisted of 118,754 patients with allergic rhinoconjunctivitis 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 with those receiving no AIT (risk reduction by 40%). Sensitivity analyses suggested significant preventive effects of SCIT with native (nonallergoid) allergens.

ADVANCES IN AIT ROUTES AND PREPARATIONS

Conventional SCIT is associated with some disadvantages, including the need for frequent injections over a minimum of 3 years, the need for visits to the doctor's office, and the risk for adverse events, including life-threatening anaphylaxis in few cases. SLIT has emerged as an alternative user-friendly approach, allowing self-administration at home and at the same time reducing the risk of severe systemic reactions. However, SLIT requires daily intake for 3 years, challenging patient adherence to the treatment. As a consequence, novel AIT approaches are constantly in development (Table I).[22-28]

These approaches aim to improve safety and patient convenience while preserving or even improving efficacy.[29] 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).[29]

Finally, the safety and efficacy of AIT can be improved when combined with other innovative treatments (eg, mAbs to IgE or T_H2 cytokines). Intradermal injections and epicutaneous applications with allergen patches have been proposed, but results have not been convincing thus far. Repeated intradermal administrations of very low doses of grass pollen allergen extract have previously been associated with suppressed allergen skin late-phase responses[30]; however, a recent RCT with preseasonal intradermal grass AIT[22] showed an increase in responsiveness and did not show beneficial effects on allergic symptoms. The efficacy of epicutaneous AIT for treatment of grass pollen–induced AR was supported previously by 2 RCTs,[31,32] but a recent trial performed by the same group[23] confirmed significant symptom improvement compared with placebo in the year of treatment but not the year after discontinuation. Hence a single course of preseasonal epicutaneous AIT was not associated with long-term efficacy.

Intralymphatic AIT involves a small number of injections of low doses of allergen directly into a lymph node. Efficacy and safety have been suggested for pollen-induced[33] and cat-induced[34] AR. An additional small cohort of patients with grass- or birch pollen–induced AR[24] also showed an improved global evaluation of seasonal symptoms. In conclusion, further assessment in well-designed, large-scale RCTs is needed before intralymphatic AIT is ready for clinical use.[35]

Investigations of allergen content and characterization of drug products used for clinical trials for OIT have been limited thus far. One study showed that peanut flour contains the major peanut allergens Ara h 1 and Ara h 2 in complete form and thus can be a viable product for peanut OIT with its low bacterial content and long shelf life.[36]

In recent years, 2 types of T-cell epitope–based allergen peptides have been developed and assessed in RCTs: short T-cell epitope peptides (also named synthetic peptide immunoregulatory epitopes) and longer contiguous overlapping peptides (COPs).[37] Previous trials with cat,[38] HDM,[39] and grass[40] synthetic peptide immunoregulatory epitope 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 3 tested dosing schemes.[25] 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 end points.[41,42]

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 with placebo was demonstrated more recently in a phase IIb trial with the lower of 2 dosing schemes.[26] 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-phase and T cell–mediated late-phase adverse events.[43] Both the safety and efficacy of this novel vaccine have been investigated in a phase II trial[27]; a single course of 3 monthly subcutaneous injections was effective in reducing allergen-induced symptoms versus 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.

TABLE I. Randomized, double-blind, placebo-controlled clinical trials in patients with AR/allergic rhinoconjunctivitis: Specific routes of administration/allergen preparations/combination of treatments

	First author, year	Product	Study population	Route of administration and dosing scheme	Study design: total no. of patients randomized	Primary outcome: *P* value
Intradermal						
Grass	Slovick et al, 2017[22]	Timothy grass pollen extract *(Phleum pratense)*	Adults (18-65 y) with grass pollen–induced AR	Seven preseasonal intradermal injections; every 2 wk; injections containing 10 BU (7 ng of the major allergen Phl p 5)	Single-center, randomized, double-blind, placebo-controlled trial (n = 93)	Daily combined symptom medication scores during the following grass pollen season, AIT vs placebo, $P = .80$ (remark: worse nasal symptoms and asthma symptoms in the active intradermal treatment group; secondary end points)
Epicutaneous						
Grass	Senti et al, 2015[23]	Grass pollen extract in petrolatum, 200 IR/mL	Adults (18-65 y) with grass pollen–induced allergic rhinoconjunctivitis	Epicutaneous; 6 patches, each applied to the upper arm and kept there for 8 h at weekly intervals, preseasonal	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, $P = .003$ compared with placebo; after treatment-free follow-up year, $P = .430$ compared with placebo
ILIT						
Grass/birch	Hylander et al, 2016[24]	Birch or grass pollen extract	Adults (18-65 y) with grass/birch pollen–induced allergic rhinoconjunctivitis	Intralymphatic injections; 3 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 how their most recent seasonal allergic symptoms were in comparison with symptoms experienced during the pollen season before treatment); $P = .047$ (remark: results not reported separately for new cohort)

(Continued)

TABLE I. *(Continued)*

	First author, year	Product	Study population	Route of administration and dosing scheme	Study design: total no. of patients randomized	Primary outcome: *P* value
Peptides Intradermal						
Grass	Ellis et al, 2017[25]	Grass allergen peptides (short T-cell epitope peptides or SPIRES)	Adults (18-65 y) with grass pollen–induced allergic rhinoconjunctivitis	Preseasonal intradermal injections; 3 regimens vs placebo: (A) 6 nmol at 2-wk intervals for a total of 8 doses "8x6Q2W" (B) 12 nmol at 4-wk intervals for a total of 4 doses "4x12Q4W" (C) 12 nmol at 2-wk 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 2-4 of a 4-d posttreatment challenge in the environmental exposure unit after the grass pollen season: (A) "8x6Q2W" vs placebo, $P = .035$ (B) "4x12Q4W" vs placebo, $P = .260$ (C) "8x12Q2W" vs placebo, $P = .452$
Peptide SCIT						
Birch	Spertini et al, 2016[26]	Bet v 1–derived peptides (COP)	Adults (18-55 y) with birch pollen–induced allergic rhinoconjunctivitis	Five preseasonal 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, $P = .015$ (B) 100 μg group vs placebo, $P = .180$
Peptide SCIT						
Grass	Zieglmayer et al, 2016[27]	Grass pollen B-cell epitope–based peptide vaccine BM32 (4 recombinant fusion proteins consisting of nonallergenic peptides derived from the IgE-binding sites of major grass pollen allergens fused to hepatitis B virus–derived PreS)	Adults (18-60 y) with grass pollen–induced AR	Subcutaneous injections; 3 injections with approximately 1-mo interval; 3 regimens vs placebo: (A) 10 μg of BM32 (B) 20 μg of BM32 (C) 40 μg of BM32	Single-center, randomized, double-blind, placebo-controlled trial (n = 71)	Difference in Total Nasal Symptom Score before and after treatment: ● Effect compared with placebo: (A) 10 μg of BM32, *P* value not given (B) 20 μg of BM32, *P* value not given (C) 40 μg of BM32, *P* value not given ● Effect compared with baseline: (A) 10 μg of BM32, $P = .102$ (−10%) (B) 20 μg of BM32, $P = .030$ (−24%) (C) 40 μg of BM32, $P = .003$ (−20%) Placebo, $P = .084$ (−17%)

(Continued)

TABLE I. *(Continued)*

	First author, year	Product	Study population	Route of administration and dosing scheme	Study design: total no. of patients randomized	Primary outcome: *P* value
Combined SCIT + anti–IL-4 Grass	Chaker et al, 2016[28]	Grass pollen extract (Alutard Avanz *Phleum*) + human therapeutic antibody to human IL-4 (VAK694)	Adults (18-60 y) with grass pollen−induced AR	Preseasonal subcutaneous AIT; 13 wk; conventional weekly updosing schedule to a suboptimal dose of 30,000 SQ + intravenous anti−IL-4 (3 mg/kg) every 4 wk (overall 4 doses) Three groups: (A) Grass SCIT + anti−IL-4 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 mo after the end of treatment, as assessed by cutaneous late-phase response to allergen; Grass SCIT + anti–IL-4 vs placebo: $P < .05$ Grass SCIT vs placebo: $P < .01$ Grass SCIT + anti–IL-4 vs grass SCIT alone: NS (+no significant difference between the groups in effect on symptoms of AR measured with visual analogue scale)

BU, Biological units; *ILIT*, intralymphatic AIT; *i.v.*, intravenous; *SPIREs*, synthetic peptide immunoregulatory epitopes.

Finally, a small single-center RCT evaluated the combined approach of suboptimal grass SCIT with monoclonal anti–IL-4 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.[28]

RECENT ADVANCES IN AIT 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 AIT have been investigated, including OIT, epicutaneous immunotherapy (EPIT), SLIT, and SCIT approaches. The recent publications of RCTs are summarized in Table II.[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 might be effective at least for the protection against accidental exposure.[45] Two dose-finding RCTs have studied the efficacy of peanut EPIT by using an allergen patch delivery system[50,51] and revealed a modest but significant treatment effect compared with 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 the safety, efficacy, and feasibility of early peanut OIT with 2 doses.[52] In both treatment arms, a high proportion of children reached sustained unresponsiveness 4 weeks after treatment discontinuation. OIT treatment in preschool children had a favorable safety profile.[52] Peanut OIT later in life showed a higher efficacy for treatment of peanut allergy compared with SLIT, but higher frequencies of adverse reactions have been reported as a disadvantage.[47] The combination of food AIT with omalizumab might further increase tolerability and efficacy and reduce the time needed for updosing, as shown for cow's milk and peanut allergy.[53,54] The use of hydrolyzed preparations for egg allergy might 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 AIT in atopic dermatitis,[56-58] no new RCTs have been published recently.

ADVERSE EVENTS AND SAFETY

A European survey on systemic adverse reactions (SARs) aimed to monitor the real-life situation by asking physicians in 3 countries, 94% of whom were allergists, about adverse events in patients undergoing AIT for pollen, *Alternaria* species, and animal dander.[59] A total of 4316 patients, more than 50% with asthma comorbidity with a follow-up of 15 months, were studied.

TABLE II. Recent developments in immunotherapy of food allergy

	First author, year	Product, manufacturer	Study population	Treatment time, route of administration, dosing scheme	Study design, total no. of patients	Primary outcome, *P* value
Peanut Epicutaneous	Jones et al, 2017[51]	Viaskin Peanut patch (epicutaneous delivery system containing dry deposit of a formulation of peanut protein extract)	Children and young adults (4-25 y) with peanut allergy	Epicutaneous patch delivery system; 52 wks; 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 wk 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, $P = .005$ (B) Viaskin Peanut, 250 μg vs placebo, $P = .003$ (45.8% in 100-μg group, 48.0% in 250-μg group, and 12.0% in placebo group)
	Sampson et al, 2015 (abstract)[50]	Viaskin Peanut patch (epicutaneous delivery system containing dry deposit of a formulation of peanut protein extract)	Children - adults (6-55 y)	Epicutaneous patch delivery system; 52 wk; 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 posttreatment 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, $P = .011$
Peanut Early OIT	Vickery et al, 2017[52]	Peanut protein OIT with 12% lightly roasted, partially defatted peanut flour	Young children (9-36 mo) with peanut allergy	Early oral immunotherapy; 12-36 mo; initial-day dose escalation, followed by build-up phase and maintenance phase; target maintenance doses of: (A) 300 mg/d (300 mg of 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 at 4 wk after discontinuing early intervention OIT (4-SU); (A) 300 mg/d arm: 85%; (B) 3000 mg/d arm: 71% over a median of 29 mo ($P = .43$ for difference between both arms; +20 of 154 matched standard-care controls were deemed OFC eligible over an average follow-up of 3.6 y: 6 of them passed the OFC; no spontaneous peanut tolerance was observed in the other control subjects → overall proportion in control group: 4%)

(Continued)

TABLE II. *(Continued)*

	First author, year	Product, manufacturer	Study population	Treatment time, route of administration, dosing scheme	Study design, total no. of patients	Primary outcome, *P* value
Peanut OIT + anti-IgE	MacGinnitie et al, 2017[54]	Peanut OIT with peanut flour (50%) + Xolair (omalizumab)	Children and young adults (7-25 y) with peanut allergy	Twelve weeks of treatment with omalizumab/placebo, followed by combined treatment with omalizumab/placebo and peanut updosing oral immunotherapy, followed by discontinuation of omalizumab (or placebo) and ongoing maintenance OIT	Single-center, randomized, double-blind, placebo-controlled trial (n = 37)	Ability to tolerate 2000 mg of peanut protein 6 wk after withdrawal of omalizumab; $P < .01$ (79% in omalizumab group vs 12% in placebo group)
Peanut OIT + probiotics	Tang et al, 2015[46]	• Peanut OIT with peanut flour (50%) • Bacterial adjuvant: *Lactobacillus rhamnosus*	Children (1-10 y) with peanut allergy	Oral administration; daily for 18 mo; initial-day dose escalation, followed by 1 injection every 2 wk updosing (8 mo), followed by maintenance phase (10 mo)	Single-center, randomized, double-blind, placebo-controlled trial (n = 62)	Sustained unresponsiveness, defined as passing both the oral food challenge at the end of OIT (T1) and after ≥2 wk of discontinuation of OIT and peanut elimination Active group (active OIT + active probiotic) vs placebo group (placebo OIT + placebo): $P < .001$ (82.1% in active group vs 3.6% in placebo group)
Peanut SLIT vs OIT	Narisety et al, 2015[47]	Peanut extract for sublingual administration and peanut powder for oral administration	Children and adolescents (6-21 y) with peanut allergy	Oral or sublingual administration; daily; initial dose escalation on first day, followed by 1/2-weekly updosing, followed by 12 mo of maintenance	Single-center, RCT (n = 21)	Induction of peanut desensitization, which was defined as a 10-fold increase in OFC threshold after 12 mo of therapy; OIT vs SLIT, $P = .76$ (64% vs 70%) Remark: Increase in median challenge dose after 12 mo of therapy: OIT vs SLIT, $P = .01$ (141-fold vs 22-fold)
Egg Hydrolyzed	Giavi et al, 2016[55]	Low-allergenic hydrolyzed egg (HydE) preparation	Children (1-5.5 y) with IgE-mediated egg allergy	Oral administration; daily for 6 mo	Single-center RCT (n = 29)	Result of an open oral food challenge at end of the treatment compared with placebo; $P = .66$ (36% in active group vs 21% in placebo group)

(Continued)

TABLE II. *(Continued)*

	First author, year	Product, manufacturer	Study population	Treatment time, route of administration, dosing scheme	Study design, total no. of patients	Primary outcome, *P* value
Egg						
OIT	Caminiti et al, 2015[48]	Dehydrated egg white	Children (4-11 y)	Oral administration; 4 mo; 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-mo randomized period of OIT with dehydrated egg white; $P < .001$ (94% in active group vs 0% in placebo group [remark: sustained unresponsiveness after 3 mo of egg avoidance was achieved in only 31% of the active group])
Cow's milk						
OIT + anti-IgE	Wood et al, 2016[53]	Nonfat dry powdered milk + Xolair (omalizumab)	Children, adults (7-35 y) with IgE-mediated cow's milk allergy	Initial 16-mo treatment with omalizumab/placebo (blinded) injections every 2 or 4 wk; open-label milk OIT started 2 wk after month 4 of omalizumab/placebo; additional 12 mo of omalizumab in active group (unblinded) and milk OIT in both groups; ± additional 8 wk 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 at month 28 (ie, end of treatment milk OIT + omalizumab) and at month 32 (ie, after 2 mo off of milk OIT) At month 28, $P = .18$ (88.9% in omalizumab group vs 71.4% in placebo group) At month 32, $P = .42$ (48.1% in omalizumab group vs 35.7% in placebo group [remark: significantly reduced treatment-related adverse reactions in omalizumab group])

OFC, Oral food challenge.

Forty-eight percent were polysensitized, and 17% had at least 1 AIT before current treatment for another allergen. About half of the products were native allergens, and the other half were allergoids; subcutaneous applications were preferred in Germany and Spain, and the sublingual route was preferred in France (mainly drops). About half of the patients received conventional updosing schemes, and the others received either cluster or rush schemes. Of the patients, 2.1% experienced at least 1 systemic reaction, and 88% of the SARs were observed in patients receiving SCIT (>57,000 applications) and 11% in patients receiving SLIT (>259,000 applications). Acute urticaria (26 cases) and dyspnea (17 cases) were only seen in patients receiving SCIT, and AR symptoms were seen in 21 patients receiving SCIT and 2 patients receiving SLIT. Seventy-six percent of the SARs occurred during SCIT updosing. Only 4 SARs were considered severe (0.07% of patients or 0.00007% of injections).

A higher risk of systemic reactions in patients receiving SCIT was associated with natural allergens versus allergoids and pollen and animal dander versus 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 favorable adverse event records for SLIT are confirmed by a pooled analysis of observations with a 5-grass tablet showing no SARs; 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 3-5 reactions according to European and World Allergy Organization classifications) and introduction of a slower updosing 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 (eg, supervision, availability of safety equipment, and access to specialized emergency services), have been recommended.[62]

MECHANISMS OF AIT

Role of mast cells and basophils in AIT

Mast cells and basophils have 2 types of effect on mechanisms of action of AIT, which can be classified as very early desensitization effects and 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 a decrease in their mediators. Both early and late effects have been shown to change during AIT, and several key articles have been published in the area. Decreased activation of mast cells and basophils can happen within a few hours in patients undergoing ultrarush venom immunotherapy; however, it takes 3 to 4 months during OIT.[63,64] Thus 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, MacGlashan and Hamilton[69] showed that CD32 affects FcεRI activity inhibition in human basophils. The function of CD32 to inhibit activation of FcεRI in human basophils suggests that measurements of IgG_2 and IgG_3 antibodies are warranted in immunotherapy studies. IgG_2 and IgG_3 appear to be the most efficacious in recruiting CD32 into interactions with antigens and antibodies on the human basophil.

One recent study indicates that human basophils might not directly respond to and be modulated by dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin (DC-SIGN) and mannose receptor–binding nonoxidized mannan-coupled allergoids because they do not express DC-SIGN and mannose receptors, unlike dendritic cells (DCs). In addition, the data suggest that if DC-SIGN and mannose receptor–binding allergens are not IgE bound, they cannot 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 AIT 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 the immunotherapy response. The basophil diamine oxidase molecule was proposed in one of the recent studies. In the SCIT, SLIT, and sublingual immunotherapy-participated 3 years (SLIT-TOL) groups percentages of allergen-stimulated diamine oxidase–positive chemoattractant receptor–homologous molecule expressed on T_H2 lymphocytes $(CRTH2)^+$ basophils were found to be greater compared with those in patients with seasonal AR. Similarly, there were lower proportions of $CRTH2^+$ basophils expressing surface $CD203c^{bright}$, CD63, and CD107a. Histamine release from basophils and serum inhibitory activity for IgE-FAB in all immunotherapy groups were found to be significantly greater compared with those from the seasonal AR group.[74] Abundance and activity of effector cells also change in response to peanut immunotherapy. 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 nonresponders.[75]

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

In another study on peanut allergy, basophils and mast cells sensitized with plasma from patients with peanut allergy but not peanut-sensitized patients showed dose-dependent activation in response to peanut. Depletion of IgG_4 from plasma of peanut allergic (and undergoing peanut OIT) children sensitized to Ara h 1 or Ara h 3 partially restored peanut-induced mast cell activation.[77]

Analysis with quantitative proteomics of pretreatment sera from patients with grass pollen allergy revealed a strong decrease in rhinoconjunctivitis symptoms after sublingual immunotherapy, which was observed in patients with high levels of O-glycosylated sialylated Fetuin-A isoforms. In addition to this, a significant upregulation of airway hyperresponsiveness, lung resistance, and T_H2 responses after allergic sensitization to ovalbumin (OVA) was observed after *in vivo* silencing of the *FETUA* gene in BALB/c mice.[78]

New findings on the role of DCs in AIT

Documenting the changes occurring in DCs during AIT is important because the efficiency of allergen-specific T-cell responses in immunotherapy directly correlates 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) reflect 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 the start of AIT.[79]

Induction of regulatory T (Treg) cells by tolerizing DC subsets is an important aspect of immune tolerance induction during AIT. Accordingly, it was observed that IL-27 secreted from mature retinoic acid–skewed DCs was important for inducing $CD25^+$ lymphocyte activation gene 3–positive, $CD49b^-$, forkhead box protein 3 $(Foxp3)^-$ Treg cells *in vitro*. Moreover, the β subunit of $IL\text{-}27^{-/-}$ $(Ebi^{-/-})$ retinoic acid–skewed DCs was ineffective in inducing tolerance to food allergens. It is suggested in this study that induction of $Foxp3^-$ Treg cells through regulatory DCs inducing immunotherapy or use of regulatory DCs themselves might be a useful strategy for tolerance against food allergies (Fig 1).[80] Additionally, a recent study has identified oral $CD103^-CD11b^+$ classical dendritic cells (cDCs) 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, a viable strategy for developing cDC-based therapeutic approaches in SLIT.[81]

Role of T cells in AIT

Suppression of effector T cells and induction of Treg cells have been one of the hallmarks of the mechanisms of AIT

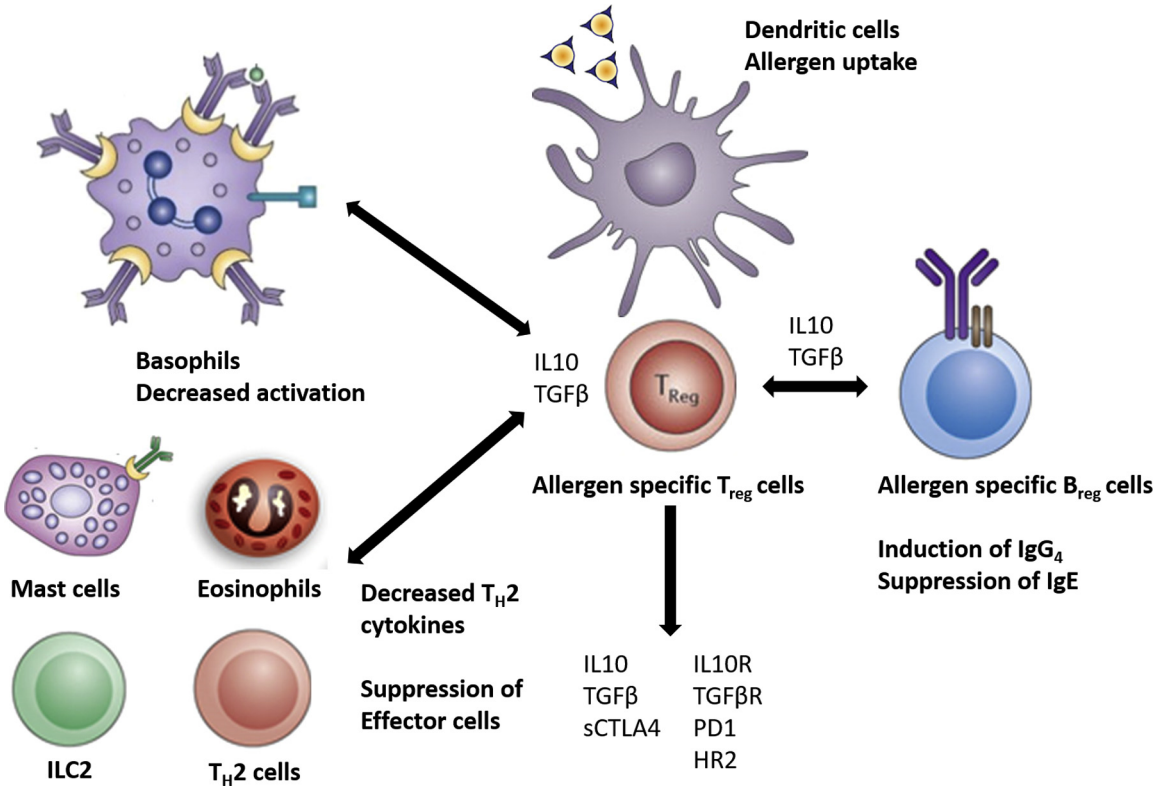

FIG 1. Cellular mechanisms of allergen-specific immunotherapy. Immune tolerance inducing conditioning and allergen uptake of regulatory DCs induces Treg and Breg cells. Allergen-specific regulatory cells induce tolerance to allergen in the periphery, and this suppressive environment regulates the effector cells of allergy. IL-10 and TGF-β production from Treg cells suppresses T_H2 cell proliferation and T_H2 cytokines while inducing IgG_4 and IL-10 from allergen-specific B cells. Absence of T_H2 cytokines in turn decreases basophil activation and mast cell, eosinophil, and ILC2 activity. *HR2*, Histamine receptor H2; *IL10R*, IL-10 receptor; *PD1*, programmed cell death protein 1; *sCTLA4*, soluble cytotoxic T lymphocyte–associated antigen 4; *TGFβR*, TGF-β receptor.

(Fig 2).[82,83] T-cell receptor (TCR) sequencing of allergen-specific T-cell clones during and after immunotherapy will mark new developments for the characterization of T cells in AIT in the near future.

A study based on next-generation sequencing of peanut-proliferative TCRβ in subjects undergoing OIT has found that the peanut allergen–induced proliferation assay leads to a polyclonal response that is extremely diverse, although 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.[84] In a cohort of participants with peanut allergy, it was observed that allergen-specific $CD4^+$ T cells expand and shift toward an "anergic" T_H2 T-cell phenotype with successful immunotherapy, a phenomenon that is not present in either pretreatment participants or healthy control subjects.[85]

Some recent studies have focused on the immunoregulatory effects of SCIT and intradermal immunotherapy and their effects on the generation of Treg cells. Production of OVA-specific IgE was decreased, but IgG_{2a} production was increased after epicutaneous immunization with OVA and CpG. Moreover, IL-4, IL-5, IL-10, and IL-13 responses and peroxidase activity of eosinophils are suppressed. Reduction of IgE synthesis is dependent on $TCR\alpha\beta^+CD4^+CD25^-$ cells, and the increase in IgG_{2a} 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 myeloid differentiation primary response gene–88, IFN-γ, and IL-17A dependent.[86] Another study on food allergy showed that epicutaneous application of antigen generated a population of gastrointestinal homing LAP^+Foxp3^- Treg cells. Mast cells were suppressed by Treg cells with the TGFβ-dependent pathway in the absence of modulation for T- and B-cell responses. These data highlight immune communication between the skin and gastrointestinal tract and identify novel mechanisms through which epicutaneous tolerance can suppress food-induced anaphylaxis.[87]

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 immunotherapy increased serum *Phleum pratense*–specific IgE levels compared with those in the control arm. T cells expanded from arm biopsy specimens of subjects undergoing intradermal immunotherapy 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.[22]

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.[88] They are in close interaction and cross-talk with T_H2 cells for T_H2 cytokine and IgE

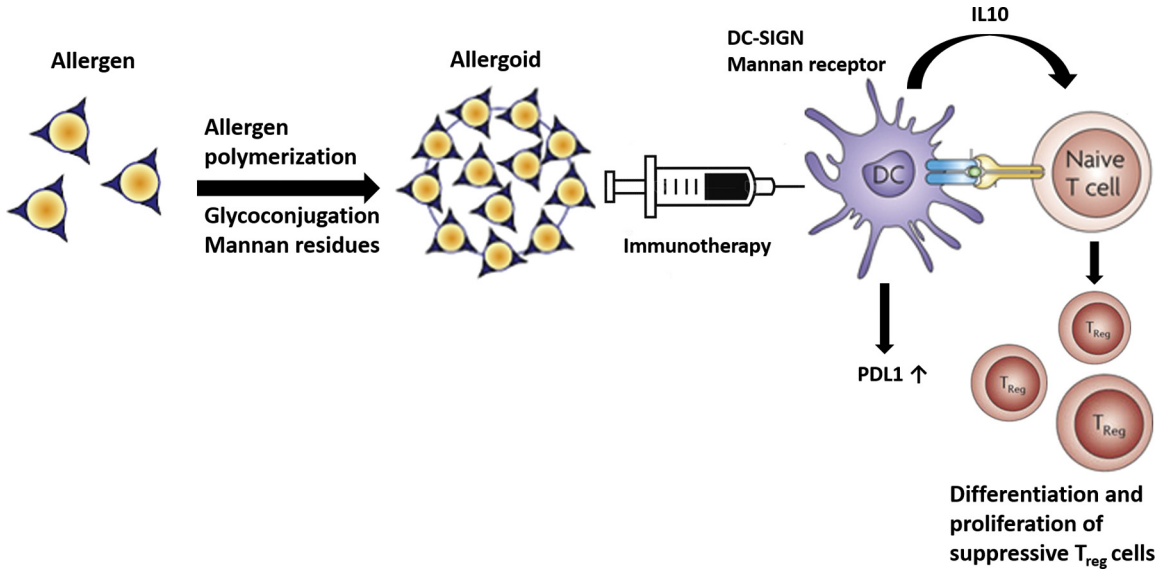

FIG 2. Mechanism of effect for mannan-conjugated allergoid vaccines in allergen-specific immunotherapy. The allergen of interest is polymerized and then glycoconjugated with mannan residues for synthesis of allergoids for vaccine use. Mannan receptor and DC-SIGN molecules expressed on human DCs facilitate uptake of allergoids and skew DCs to a regulatory phenotype. IL-10 secretion, together with presentation of allergoid-derived peptides, drives the allergen-specific naive T-cell pool into differentiation as Treg cells. PDL1 on DCs plays an important role in this context.[83]

production.[89] Type 2 innate lymphoid cells (ILC2s) are dominant in allergic patients in the circulation, and affected tissues might play a role in continuation of the allergic status and resistance to AIT.[90] IL-33–stimulated ILC2s block the generation of allergen-specific Treg cells and favor food allergy through production of IL-4 in mice. These findings suggest that blocking the IL-33/IL-33 receptor pathway or suppressing general ILC2 activation represents an innovative approach for the treatment of food allergy.[91]

Circulation and presence of ILC2s in the peripheral blood system and affected tissues of allergic patients might have a role in continuation of the allergic status and resistance to AIT.[90]

Recent reports on B-cell regulation in AIT

During the last few years, it appeared that B-cell regulation is equally important as T-cell regulation in AIT. Human regulatory B (Breg) cells, particularly their IL-10 production, were demonstrated to increase during AIT.[92,93] Peanut OIT induces an early and transient expansion of circulating Ara h 2–specific memory B cells that peaks at week 7. By 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 and somatically hypermutated and shares similar clonal groups among unrelated subjects consistent with convergent selection.[94] Supporting this concept, liposomes simultaneously targeting CD22 and the B-cell receptor specific for the major peanut allergen Ara h 2 can be used to prevent sensitization to Ara h 2. Based on previous studies with other antigens, it can be hypothesized that simultaneous engagement of CD22 and the Ara h 2–specific B-cell receptor leads to deletion of 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.[95] In addition to these, most peanut allergen–specific B cells express mutated and class-switched antibodies. After immunotherapy, 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.[96] However, in another study peanut-specific binding patterns were very similar for IgE and IgG_4. This might indicate that the IgE and IgG_4 antibodies are clonally related.[97] Use 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/IL-10 through a 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/IL-10 can be used as a novel therapeutic tool for nasal allergy.[98]

Recent studies have added new biomarkers for detecting and targeting Breg cells for controlling allergic inflammation. Selb et al[99] showed that casein- and food-induced allergic responses in mice can be controlled by IL-10–producing $CD5^+$ Breg cells. The population of IL-10–producing $CD5^+$ B cells showed an increase in mesenteric lymph nodes but not in spleens or the peritoneal cavity in OT mice. Casein-induced allergic responses were suppressed by adoptive transfer of $CD5^+$ B cells from mesenteric lymph nodes in an allergen-specific and IL-10–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 IL-10–producing Breg cells control food allergy, and mesenteric Breg cells can potentially act as a therapeutic regulator for food allergy.[100] Furthermore, CD23 surface density

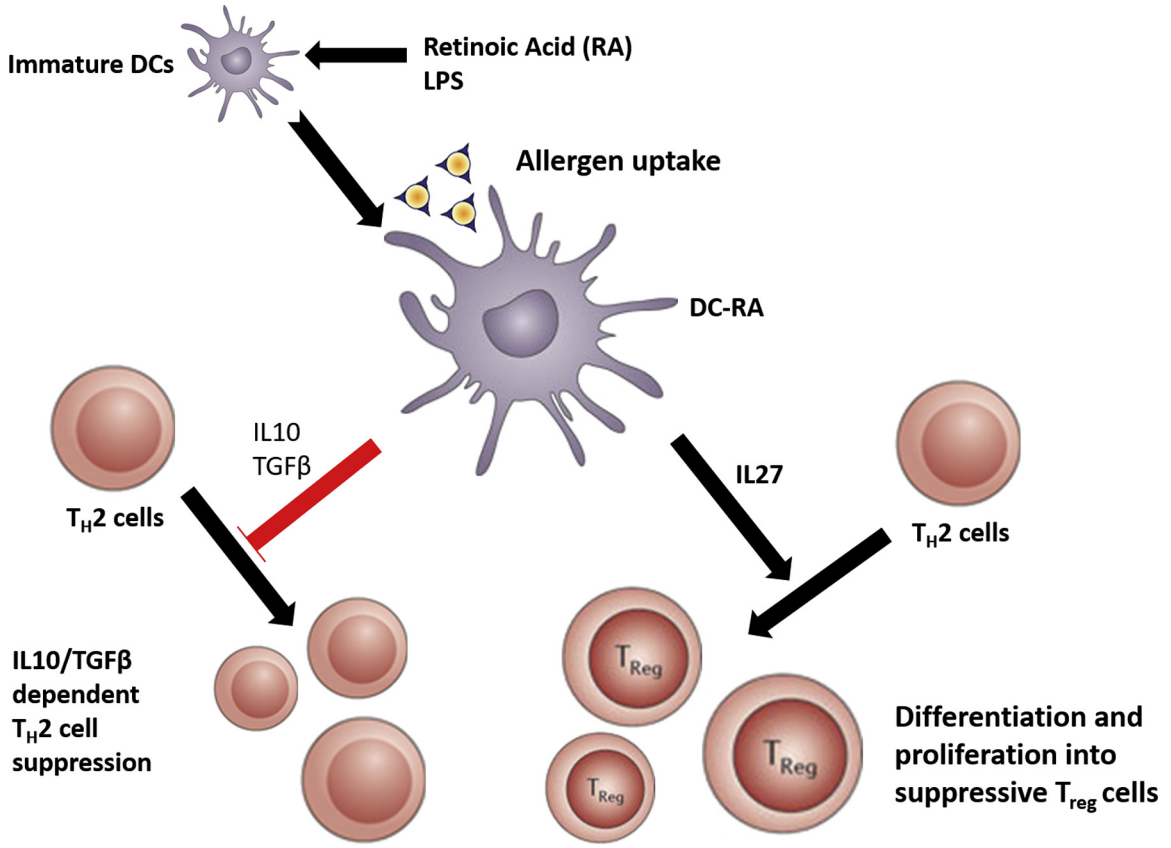

FIG 3. Mechanism of effect for retinoic acid supplementation in allergen-specific immunotherapy. Retinoic acid and LPS-pulsed human DCs together with uptake of allergen become retinoic acid–skewed dendritic cells *(DC-RA)*. These DCs with a regulatory phenotype are capable of secreting IL-10, TGF-β, and IL-27. IL-10 and TGF-β in turn suppress proliferation and cytokine secretion from T_H2 cells, whereas IL-27 drives T_H2 and naive T cells to differentiate as Treg cells, establishing tolerance to allergen in the periphery.

on B cells of allergic patients correlates with allergen-specific IgE levels and determines allergen uptake and subsequent activation of T cells. This might open new possibilities for controlling T cell-mediated allergic inflammation by targeting this pathway.[99]

Suppressive and tolerogenic B-cell frequencies are greater after bee venom immunotherapy and in patients with occupations with regular allergen exposure, such as beekeepers. In the study by Boonpiyathad et al,[101] allergic patients and beekeepers showed increased frequencies of plasmablasts, phospholipase A (PLA)–specific memory B cells, and IL-10–secreting $CD73^{-}CD25^{+}CD71^{+}$ B_R1 cells. After exposure to bee venom, PLA-specific IgG_4-switched memory B cells were observed to expand. PLA-specific B cells also showed increased expression of CCR5 after high-dose allergen exposure, whereas CXCR4, CXCR5, CCR6, an CCR7 expression was unaffected.[101]

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

The efficacy of sublingual tablets in patients undergoing 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 immunotherapy tablet MK-8237 (Merck/ALK-Abelló, Hørsholm, Denmark), 6 DU of MK-8237, or placebo daily for 24 weeks. MK-8237 with a dose of 12 DU reduced nasal and ocular symptoms. Specific IgE and IgG_4 levels increased with the 12- and 6-DU treatments versus placebo at week 8, and significant increments with IgE and IgG_4 levels were observed at week 24.[12]

In another study adults with HDM-associated AR were given a daily placebo tablet or sublingual immunotherapy tablet (STG320) for 6 months. At study entry, serum levels of *Dermatophagoides pteronyssinus*– and *Dermatophagoides farinae*–specific IgE and IgG_4 were similar across treatment groups. Within the first 2 treatment months, mite allergen–specific serum IgE levels increased 5- to 7-fold in the active treatment group and then gradually decreased while remaining unchanged in the placebo group. Mite allergen–specific serum IgG_4 levels increased over the treatment period in the active treatment group and showed a small change in the placebo group.[13]

Two studies that monitored IgG_4 responses during birch pollen immunotherapy have reported that Bet v 1–specific IgG_4 repertoires induced by birch pollen extract AIT do not broadly expand in the course of treatment in most of the patients. IgG_4 diversity increased in 20% of patients with prolonged therapy.[102] A second study applied a chimera-based approach to monitor development of the Bet v 1–specific IgE, IgG_1, and IgG_4 repertoires during 3 years of AIT in subjects. In most patients treated with allergen chimera peptides, Bet v 1–specific IgE levels

increased during the early phase of treatment, followed by a gradual decrease. All patients had Bet v 1–specific IgG_4, and 64% of patients had Bet v 1–specific IgG_1. In these subjects Bet v 1–specific IgG_4 levels increased later than IgG_1 levels. The data suggest that allergen-specific IgE and AIT-induced IgG_4 and IgG_1 repertoires differ between each other but did not expand over time. Both IgG_1 and IgG_4 induced during AIT displayed more restricted epitope diversities than IgE antibodies.[103] Furthermore, Bet v 1–derived molecules containing COPs were shown to lead to a significant increase in serum Bet v 1–specific IgG_4 levels in all but a few patients. Specific IgG_4 levels were significantly increased compared with placebo in both treated groups after treatment, as well as during and after the pollen season.[26]

Studies monitoring the efficacy of egg OIT concluded sustained unresponsiveness (a clinical remission with immunologic effects) of the immune system to the allergen persists after cessation of long-term egg OIT. Children with egg allergy received egg OIT for up to 4 years or placebo for 1 year or less. Over time, egg-specific IgG_4 levels were significantly greater in the sustained unresponsiveness group. Similarly, scores of egg-specific skin prick tests were observed to be significantly lower in those achieving sustained unresponsiveness. For egg IgE levels and basophil activation, there was a decrease over time in those achieving sustained unresponsiveness; however, the differences were not significant.[104] For introduction of egg early in life, a recent study found that the IgG_4 response to egg proteins and IgG_4/IgE ratios were found to be higher in children introduced to egg protein at 12 months. Introduction of whole egg powder into the diets of high-risk infants reduced sensitization to egg white and induced egg-specific IgG_4. However, 8.5% of infants exposed to egg protein were not susceptible to this primary prevention.[105] In addition, increased levels of egg white–specific IgA and IgA_2 were found biomarkers for clinical response to egg OIT.[106]

In another study food allergy–prone IL-4raF709 mice treated with specific IgG at the beginning of feeding showed prevention of IgE antibody, T_H2 response, and anaphylaxis development on challenge. When given as a supplement to oral desensitization in mice with established IgE-mediated hypersensitivity, IgG antibodies facilitated re-establishment of tolerance, probably because of favoring the expansion of $Foxp3^+$ Treg cells 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.[67]

Predominant Api m 10 sensitization was shown to be a risk factor for treatment failure in patients undergoing honeybee venom (HBV) immunotherapy. In patients with HBV allergy who underwent controlled honeybee sting challenge after at least 6 months of bee venom immunotherapy, no differences were observed between responders and nonresponders regarding levels of IgE sensitization to Api m 1, 2, 3, and 5. In contrast, Api m 10–specific IgE levels were moderately but significantly increased in nonresponders. Specific IgG_4 induction to Api m 10 was observed only in patients unresponsive to bee venom immunotherapy. Results from allergic patients suggest that predominant IgE sensitization to Api m 10 might be an increased risk factor for treatment failure in patients undergoing HBV immunotherapy.[107]

Several recent studies were directed toward investigating changes in allergen-specific IgE and IgG_4 responses during peanut immunotherapy and how different methods of immunotherapy affect IgE and IgG_4 levels during and after peanut immunotherapy. 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 with matched control subjects. Allergic side effects during early OIT were common, but all were mild to moderate.[52]

Uotila et al[108] reported that specific IgE to Ara h 2 and 6 decreased significantly during OIT. Although baseline measurements showed low amounts of specific IgG_4 to Ara h 1, 2, 3, 6, 8, and 9 and whole peanut extract, specific IgG_4 levels to these allergens significantly increased during OIT. OIT resulted in increased IgG_4/IgE ratios for Ara h 1, 2, 3, and 6 and whole peanut extract, whereas those for Ara h 8 and 9 remained stable. The strongest correlations were observed for Ara h 2, 3, and 6 and whole peanut extract. The study concluded that serologic response to peanut allergens during OIT is directed toward 2S albumins and peanut storage proteins.

Burk et al[109] 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. Another study confirmed these findings by showing increases in peanut-specific IgG_4 levels and IgG_4/IgE ratios in peanut EPIT–treated participants, along with trends toward reduced basophil activation and peanut-specific T_H2 cytokine levels.[51]

The Epidemiologic Study on the Genetics and Environment of Asthma, Bronchial Hyperresponsiveness, and Atopy investigated the effects of route of exposure to allergens by analyzing IgE and IgG responses 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 the dose and route of exposure and overall immunogenicity of the allergen. Allergen contact through the oral route might preferentially induce IgG responses.[110] A multicenter birth cohort study testing IgG and IgG_4 responses against 91 allergenic molecules reported that the route of allergen exposure and initial status of IgE sensitization were profoundly important to the children's repertoire to foodborne and airborne allergens at 2 years of age, whereas the amount of IgG_4 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

Adjuvanticity of certain allergen modifications and novel adjuvants represent an important part of novel vaccine approaches, and related innate mechanisms might define efficacy in patients receiving AIT.[112] New findings for increasing efficacy for allergoid immunotherapy show that low-dose therapy with a chemically modified monomeric allergoid of Der p 2 alone was not fully successful, but supplementation of vitamin D_3 was associated with limited changes in the immunologic parameters in the lung. In contrast, the most prominent decrease in airway eosinophilia and T_H2 cytokine levels and the concomitant increase in Treg cell counts, IL-10 levels in the lung, and Der p 2–specific IgG_{2a} levels in serum were induced by allergoid vaccine adjuvanted with vitamin D_3.[113]

Characterization and peptide sequencing of major allergen types and exploitation of their structure for recombinant peptide-based AIT might be a viable strategy for increasing the

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, has been shown to have a lower effect on lymphocyte proliferation compared with grass pollen extract, and BM32 also induced significantly lower secretion of proinflammatory cytokines.[115]

Another study with the 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 use as allergen vaccines. Human DCs can capture glutaraldehyde-polymerized allergoids conjugated to nonoxidized mannan, which display *in vivo* hypoallergenicity, much more efficiently than native grass pollen *P pratense* allergens or mannan-free glutaraldehyde-polymerized allergoids. Uptake of mannan-conjugated allergoids depends on mannose receptor and DC-SIGN–mediated internalization. Nonoxidized mannan allergoid skews human DCs to generate functional $Foxp3^+$ Treg cells through programmed death ligand 1. Immunization of mice with nonoxidized mannan induces a shift to nonallergic responses and increases the frequency of splenic $Foxp3^+$ Treg cells (Fig 3).[83,117]

New studies on conjugated allergoids show efficacy in control of inflammation in patients with various allergic diseases. A recent study showed that numbers of eosinophils in bronchoalveolar lavage fluid and lung tissue, total IgE levels, and production of IL-13 from lung mononuclear cells all decreased significantly in the BALB/c mice challenged and treated with Toll-like receptor 7 ligand–allergen conjugates in comparison with nDer p 2–treated mice. nDer p 2–conjugated stimulated spleen cells showed a significant increase in IFN-γ and IL-10 levels, as well as IgG_{2a} levels.

Similar effects were elicited by treatment with OVA conjugate in an OVA-driven BALB/c model. Cytofluorometric analysis demonstrated that the conjugate expanded IFN-γ–and IL-10–producing memory T cells. IL-$10^{-/-}$ and IL-$12^{-/-}$ mice were used to confirm the role of IL-10 and IFN-γ in inducing a protective and balanced redirection the T_H2-mediated airway inflammation.[118] In another study milk-sensitized mice undergoing 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 undergoing EPIT completely prevented sensitization to peanut and peanut-induced anaphylaxis. Milk EPIT also enhanced methylation of the GATA-3 promoter region.[119]

Targeting the complement system might become a new strategy for increasing the 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 IL-10–secreting $CD4^+$ T cells with suppressive activity. Furthermore, C1q expression is upregulated in PBMCs 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.[120]

AIT combined with biological agents and probiotics

Studies on the combination of biological agents with AIT seemed more and more frequent in recent years. A combined therapy comprising a probiotic together with peanut OIT was recently reported. Cotreatment of the 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 IgG_4 levels. This is the first RCT assessing the novel coadministration of a probiotic and peanut OIT and evaluating sustained unresponsiveness in children with peanut allergy.[46]

Similarly, for milk immunotherapy, combining omalizumab therapy with milk OIT led to distinct alterations in basophil reactivity but not T-cell responses.[121] According to the same concept, a multicenter RCT study has shown that long-term use 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.[122] 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 the omalizumab-treated group at the 4th month and then reduced at the 32nd month. In the omalizumab-treated group percentages of $CD63^+$ basophils were found to be lower compared with those in the placebo group at the 28th month. Afterward, at the 32nd month, the percentage of $CD63^+$ basophils in the omalizumab-treated group reached levels similar to those in the placebo group.[53]

Short-term grass pollen SCIT under the umbrella of anti–IL-4 was investigated in a recent study. Treatment with anti–IL-4 and SCIT compared with SCIT alone induced decreased allergen-specific IL-4–producing cell counts. Dual IL-4/IL-10–producing cells were induced in both active treatment arms during the pollen season. There was no additional benefit of the combination of anti–IL-4 with SCIT over SCIT alone.[43]

CONCLUSION

Recent developments in the clinics and cellular and molecular mechanisms of AIT aim at enhancing clinical and immunologic tolerance, decreasing side effects, and increasing efficacy. Several clinical trials have been completed successfully during the last couple of years, reaching an end point. Hypoallergenic recombinant allergen and allergoid vaccines and use of probiotics, vitamins, and biologic agents 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 IgG_4 and several key molecules that can act as biomarkers are continuously being developed

(see Table E2 in this article's Online Repository at www.jacionline.org). As new technologies and novel strategies emerge, we are in need of more research into the mechanisms, biomarker discovery, and disease phenotyping for AIT. AIT represents the most common and one of the very few human *in vivo* relevant antigen-specific immune tolerance models, and there will always be take-home messages for other immune tolerance–related conditions, such as autoimmunity, organ transplantation, chronic infections, cancer, and recurrent abortions.

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