



Novel immunotherapy approaches to food allergy

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Purpose of review

Despite reaching high percentages of desensitization using allergen-specific immunotherapy (SIT) in patients with food allergy, recent studies suggest only a low number of patients to reach persistent clinical tolerance. This review describes current developments in strategies to improve safety and long-term efficacy of SIT.

Recent findings

Modified allergens or tolerogenic peptides, ultimately optimized for human leukocyte antigen background of the patient, are explored for tolerance induction, whereas anti-IgE antibody (Omalizumab) may be used to facilitate SIT safety. Adjunct therapies to enhance efficacy may make use of T_H1 polarizing agents, for example, CpG-oligodeoxynucleotides combined with modified allergen packaged in nanoparticles. Preclinical studies showed insulin-like growth factor-2, intravenous immunoglobulin, Tregitopes or allergen encased oligomannose-coated liposomes capable of inducing regulatory T-cells, recognized for their importance in clinical tolerance induction. Dietary intervention strategies utilizing herbal formula 2, VSL#3, nondigestible short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides (scGOS/lcFOS) plus *Bifidobacterium breve* M-16V or n-3 long-chain polyunsaturated fatty acids may facilitate safety and/or a favourable milieu for tolerance induction.

Summary

Combining SIT using (adapted) allergens or tolerogenic peptides with adjunct therapy may be essential to improve safety and/or efficacy. Beyond using targeted approaches, specific dietary components may be explored to reduce side-effects and support clinical tolerance induction by SIT.

Keywords

adjunct therapy, clinical tolerance, desensitization, food allergy, immunotherapy

INTRODUCTION

The rising prevalence of allergic diseases relates to the increase in noncommunicable diseases, and food allergy may be seen as an early onset non-communicable disease [1]. Food allergy is one of the first manifestations of atopic constitution and affects 6% of children and 3 to 4% of adults in westernized countries. Cow's milk and hen's egg are the main contributors to early childhood allergy and are outgrown in 80% of patients, by contrast, peanut allergy is resolving in less than 20%. Intake of the culprit food results in local (oropharyngeal/gastrointestinal) and/or systemic (atopic dermatitis, asthma) symptoms, ranging from mild (itching) to extremely severe (anaphylactic shock). Food allergen-specific immunotherapy (SIT) is being explored, but safety and long-term efficacy issues currently question its applicability for general clinical practice [2,3]. This review provides an update on clinical trials published over the last year using SIT in IgE-mediated cow's milk, hen's egg and peanut allergy.

Furthermore, novel developments in allergen modification and adjunct therapies to improve safety and (long-term) efficacy are discussed.

MECHANISM OF ALLERGY AND IMMUNOTHERAPY

Food allergy results from a defect in the adaptive immune response upon presentation of food

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KEY POINTS

- Specific OIT for egg, milk or peanut allergy results in desensitization in the majority of cases.
- Tolerogenic peptides from allergen sequences having low allergenic properties are being selected.
- Omalizumab may enhance SIT safety; DARPin are novel effective desensitizing agents.
- Supporting allergen-specific functional Treg may pave the way for clinical tolerance induction.
- Dietary FAHF-2, VSL#3, scGOS/lcFOS or n-3 LCPUFA may favour the immune milieu for SIT.

proteins by antigen-presenting cells to T-cells resulting in T_H2 polarization and IgE-producing plasma cells rather than tolerance [3]. Allergen-specific IgE binds fragment crystallizable epsilon receptor 1 (Fc ϵ RI) on mast cells and basophils (sensitization) and allergen reencounter cross-links membrane-bound IgE triggering degranulation, resulting in allergic symptoms [3]. Immunotherapy involves desensitization ultimately by reducing antigen-specific IgE and internalization of membrane-bound allergen-specific IgE [4], while increasing IgG4. Immunotherapy also aims at restoring tolerance by supporting T_H1 and regulatory T-cells (Treg) (CD4⁺CD25^{high}FoxP3⁺, Tr1 or T_H3 cells). Treg suppress T_H2 -prone immune responses via secretion of IL-10 and/or TGF- β [5,6[■],7[■]]. Indeed, allergen-induced Treg were recently found to associate with reestablishment and maintenance of clinical tolerance using SIT [6[■]].

ALLERGEN-SPECIFIC IMMUNOTHERAPY FOR FOOD ALLERGY

SIT for food allergy utilizes conventional or rush (within days) allergen dose-escalation protocols followed by daily home maintenance with the tolerated threshold dose. Dose escalation aims at desensitizing, increasing the threshold dose for clinical reactivity to the culprit food. Maintenance aims at reaching (persistent) clinical tolerance (no clinical symptoms for the threshold dose after ceasing therapy for at least 4 weeks) [2,3]. For food allergy, oral immunotherapy (OIT) and sublingual immunotherapy (SLIT) are most often studied [2,3]. OIT lowers the relative risk ratio for reactions to 0.19 as a result of desensitization [8]. However, only in a few studies [6[■],9[■]], clinical tolerance after ceasing therapy was determined and results are disappointing. Apart from OIT and SLIT, also subcutaneous immunotherapy was shown effective in, for example,

peanut allergy, but coincided with a high frequency of severe side-effects [3]. Novel approaches include epicutaneous immunotherapy (EPIT).

Oral immunotherapy

Several recent studies [6[■],10,11,12[■],13] for peanut allergy showed desensitization in patients undergoing OIT. Anagnostou *et al.* [10] found 62% desensitization allowing an eliciting dose threshold increase of 25 times compared with the placebo group, but 20% of OIT patients experienced respiratory difficulties and in one case, epinephrine was administered. These adverse effects are not uncommon for OIT and pose a serious drawback (Table 1). Immunological changes required to reach a state of clinical tolerance rather than desensitization are largely unknown. Recently, OIT in peanut-allergic patients increased the frequency of highly suppressive antigen-induced forkhead box P3 (FoxP3) expressing Treg in tolerant participants. Only 13% of all participants in the OIT group remained unresponsive after 6 months of peanut avoidance, but persistent hypomethylation of the FoxP3 locus was associated with maintaining clinical tolerance [6[■]]. Keet *et al.* [9[■]] also found that persistent clinical tolerance was only reached in 31% of patients undergoing OIT in cow's milk allergy. It was suggested that the duration of OIT plays a role in sustained unresponsiveness [14]. OIT is a common method for inducing desensitization in hen's egg-allergic children and raw egg extract desensitized 80–90% [15,16]. Current recruiting clinical trials are implementing baked egg or egg protein to desensitize patients [17].

Sublingual immunotherapy

SLIT is extensively investigated as potential therapy for food allergies [12[■],18[■]]. Fleischer *et al.* [18[■]] showed desensitization in 70% of participants in a peanut SLIT trial compared with placebo controls. Adverse effects mostly included mild oral-pharyngeal symptoms [18[■]]. Another recent study found OIT to a greater extent to induce immunological changes, such as lowering the peanut IgE over IgG4 ratio, than SLIT. Also, dose-eliciting thresholds were higher and more stable in OIT-treated patients than SLIT-treated patients [12[■]]. Although the efficacy of SLIT is lower compared with OIT, its safety profile could be beneficial for further clinical trials [12[■],19].

Epicutaneous immunotherapy

EPIT can induce effector responses via Langerhans cells, which are activated by a skin-patch containing

Table 1. Summary of recent clinical trials for oral immunotherapy, sublingual immunotherapy and epicutaneous immunotherapy

Reference	Allergen/therapy	Number of patients	Desensitization/tolerance	Immunological changes	Safety
Anagnostou 2014	Peanut; OIT	85 (ages 7–16) Placebo: 46; OIT: 39	Desensitization; Full: 62%; Partial: 22%; Increased median threshold 25-fold	Not investigated	Epinephrine given to one patient subject. 19% β 2-agonist
Chin 2013	Peanut; OIT/SLIT	50 (ages 2–11); OIT: 23; SLIT: 27	OIT three times more likely to pass DBPCFC at 12 months. Variable and lower eliciting dose thresholds by SLIT	OIT larger changes in peanut IgE and IgG4 levels than SLIT	Four doses epinephrine for two patients in OIT
Fleischer 2013	Peanut; SLIT	40 (age 12–37); Placebo: 20; SLIT: 20	Desensitization SLIT: 70%; Placebo: 15%	Increase allergen-specific IgG4 for SLIT patients. No difference responders and nonresponders	Dose-related oral-pharyngeal symptoms; One dose epinephrine
Syed 2014	Peanut; OIT	43 (ages 4–55); OIT: 23; Abstain- ing peanut: 20	Desensitization; OIT: 87%; Lasting tolerance: 13%	Immune-tolerant patients higher antigen- induced Tregs. IgG4 no predictor of lasting tolerance	Not assessed
Vickery 2014	Peanut; OIT	39 (ages 1–16)	Sustained unresponsiveness: 31% (1 month after OIT)	Sustained unresponsiveness correlated with lower baseline IgE levels. No role for IgG4	15% withdrawal allergic side-effects
Dello Iacono 2013	Hen's egg; OIT	20 (5–11 years); OIT: 10; Placebo: 10	Desensitization; Partial: 90% (median 20 ml)	Increase threshold not correlated with level serum-specific IgE	Adverse effects in all subjects; patients, dose-related. 66% grade III, 9% grade IV
Meglio 2013	Hen's egg; OIT	20 (age $\geq$ 4 years)	Desensitization; Full: 80% (25 ml); Par- tial: 10%	IgG4 levels for ovalbumin increased significantly	30% reached full dose symptom free
Keet 2013	Cow's milk; OIT	32 Follow-up from two studies	Follow-up study, 31% full serving with minimal or no symptoms	Baseline IgE important for outcome	19% at least one anaphylaxis, 9% at least one dose of epinephrine
Vázquez-Ortiz 2013	Cow's milk; OIT	81 (age 5–18)	Desensitization; Full: 72% (200 ml); Partial: 21%	Baseline IgE important for outcome	14 patients persistent reactions; six adverse effects

OIT, oral immunotherapy; SLIT, sublingual immunotherapy.

allergenic proteins [19]. Current phase I/II trials for peanut allergy are ongoing; so far, results regarding safety seem promising [19]. An EPIT trial in peanut-sensitized mice demonstrated the contribution of Treg to desensitization, which maintained their suppressive capacity after treatment was completed [20].

ALLERGEN ADAPTATION TO IMPROVE SAFETY AND/OR EFFICACY

Adaptations of food allergens to reduce allergenicity, while retaining their capacity to induce desensitization or tolerance induction, can improve safety and efficacy of SIT. Allergenic proteins in cow's milk and hen's egg comprise conformational heat labile [e.g. β -lactalbumin, ovalbumin (OVA)], as well as sequential heat stable [e.g. α_{s1} -casein, ovomucoid (OVM)] IgE-binding epitopes [21]. Hence, heating will not completely reduce the allergenic risk, and in a recent study [22], it was shown that 64% of egg-allergic children could tolerate baked egg. The heating process may influence tolerogenic properties of the allergenic protein as was tested in mice expressing T-cell receptors (TCRs) specific for dominant T-cell epitope OVA_{323–339}. Unheated egg white, 80 or 121°C heated egg white enhanced the allergenicity or immunogenicity, whereas 100°C heated egg white showed reduced allergenicity while maintaining tolerogenic capacities [23]. Indeed, in OVM-sensitized mice, desensitization via OIT was equally effective using the native or heated OVM, and introduction of baked egg in the diet of egg-allergic patients resulted in desensitization for raw egg in 50% of cases [24,25].

PEPTIDE IMMUNOTHERAPY

As an alternative for (adapted) allergen treatment, an increasing amount of encouraging preclinical data is published on peptide SIT for food allergy [26]. OIT using OVM immunodominant T-cell epitope-containing peptides reduced the frequency of clinical signs and allergen-specific IgE in OVM-allergic mice, while increasing Treg frequency in the circulation [27]. T-cell epitopes as candidates for peanut allergy SIT are selected on the basis of proliferation of CD4⁺ T-cell lines generated from peanut-allergic patients, on basophil activation assays identifying the non-IgE cross-linking peptides and human leukocyte antigen (HLA)-genotyping [28,29]. A recent genome-wide association study [30^{***}] in 5.789 allergen-sensitized individuals versus 10.076 controls showed, amongst others, that sensitization is associated with HLA-B and –DQ beta (DQB)1. T-cell responses towards

allergens are initiated by allergen internalization and degradation followed by peptide presentation in the context of HLA molecules. Cognate T-cell help is required for isotype class switching and induction of antigen-specific memory B cells and plasma cells [31]. Specific HLA molecules exhibit distinct peptide specificities; thus, the responsiveness of individuals to epitopes within allergens can theoretically be predicted per individual, grouped according to their major histocompatibility complex background. By using NETMHCII algorithms, a number of Ara h 2 peptides binding to histocompatibility complex class II were identified and proven to be functional as similar peptides were shown to induce T-cell proliferation [32]. In a direct approach to identify Ara h 1-specific peptides, T-cell stimulation with 20-mer overlapping peptides from different individuals resulted in 145 Ara h 1-specific T-cell clones. HLA restriction analysis showed HLA-DQ and –DR as presenting molecules for different peptides defining apparently without a background preference for specific (e.g. DR beta 1*03:01) alleles [29]. Prickett *et al.* [29] designed seven short peptides containing 10 core T-cell epitopes of Ara h 1 and three peptides containing five core T-cell epitopes of Ara h 2 [28]. It is suggested that a potential peptide immunotherapy with the combined Ara h 1 and Ara h 2 peptides targets a HLA-diverse patient population; however, preclinical and clinical studies still need to examine the therapeutic potential of those peanut peptides. In cow's milk allergy, T-cell epitopes have been identified for both beta-lactoglobulin [33^{*}] and alpha-lactalbumin [34]. Oral delivery of two specific beta-lactoglobulin T-cell epitope peptides prior to whey sensitization reduced the development of the whey-induced acute allergic skin response in mice [33^{*}]. Innovative approaches for peptide immunotherapy aim at enhancing the tolerogenic properties, for example, by adding sugar moieties suggested to induce Treg by activating C-type lectin receptor specific intercellular adhesion molecule 3 (ICAM-3) grabbing nonintegrin-related 1 on tolerogenic dendritic cells [35,36].

MUCOSAL VACCINES

Approaches using allergen delivery to mucosal surfaces via nanoparticles or bacterial vectors are under development. Recently, EMP-123, a rectally administered suspension of killed *Escherichia coli* expressing encapsulated modified recombinant Ara h 1, Ara h 2, Ara h 3 and shown effective in mice, induced severe acute side-effects, including two anaphylactic reactions in five out of 10 peanut-allergic patients [37]. An alternative for the use of *E. coli* as a vector may be the *Lactococcus lactis* [38]. Prophylactic oral

delivery of *L. lactis* engineered to secrete Ara h 2 or have cell wall anchored Ara h 2 reduced peanut-specific IgE and T_H2 polarization in peanut-sensitized mice [38].

ADJUNCT TREATMENT ADDED TO SIT TO ENHANCE DESENSITIZATION

Humanized anti-IgE antibody Omalizumab binds free IgE and dissociates IgE from the surface of mast cells, consequently reducing FcεRI expression [39,40]. Combining SLIT or OIT with Omalizumab seems promising as in a pilot study in high-risk peanut-allergic patients rapid oral desensitization was reached with only mild adverse effects, while monotherapy with Omalizumab resulted in high rates of severe side-effects in patients with peanut allergy [2,39,40]. Designed ankyrin repeat proteins (DARPin) also prevent binding of free IgE and DARPin bi53_79 completely dissociated IgE from FcεRI and blocked cutaneous anaphylaxis in mice expressing human FcεRIα chain better than Omalizumab [39]. Hence, beyond anti-IgE antibodies, in future DARPin may be added to SIT as adjunct therapy to improve safety (Fig. 1).

TH1 INDUCING CPG-OLIGODEOXYNUCLEOTIDES

Toll-like receptor 9 agonists, for example, bacterial cytosine phosphate guanine (CpG) DNA rich in non-methylated cytosine guanine motifs or synthetic CpG-oligodeoxynucleotides (ODN), induce T_H1 polarization. Oral delivery of digestion-protected CpG-ODN-treated allergic symptoms in peanut-sensitized mice [41], type B and C CpG-ODN (i.p.) proved to be most effective [42]. In a phase I/IIa trial with house dust mite-allergic asthma patients QbG10 (bacteriophage Qbeta-derived virus-like particle with CpG-motif G10 inside) containing type A CpG as adjuvant in allergen-specific subcutaneous immunotherapy, effectively relieved symptoms lasting for 38 weeks after treatment [43]. QbG10 without allergen treatment also reduced asthmatic symptoms and improved the forced expiration volume up until 12 weeks after steroid withdrawal [44]. Combined injection of Ara h 2 with CpG-ODN packaged in biodegradable protamine (proticles) suppressed generation of Ara h 2-specific IgE in BALB/c mice [45]. Indicating nanoparticles as a novel way of combined delivery of allergens and adjuvant in SIT.

REGULATORY T-CELL INDUCING STRATEGIES

Apart from enhancing T_H1 polarization, allergen-specific Treg may improve SIT efficacy (Fig. 1). Recently, the insulin-like growth factor-2 (IGF-2) receptor was found to be expressed on

CD4⁺CD25⁺FoxP3⁺CD127⁻ Treg in mouse and human [7^{***}]. IGF-2 induced proliferation of functional Treg upon concurrent TCR activation, while preventing oral OVA sensitization in mice [7^{***}]. Intravenous immunoglobulins (IVIg) (polyclonal IgG) provide another way of enhancing Treg numbers. The sialic acid fraction of IVIg instructs tolerogenic dendritic cells via binding of dendritic cell immunoreceptor. In-vivo treatment with sialic acid IVIg (i.p.), as well as adoptive transfer of OVA primed ex-vivo sialic acid IVIg exposed dendritic cells, prevented OVA-induced airway hyperresponsiveness in mice, while enhancing pulmonary Treg frequency [46^{***}]. Furthermore, HLA class II restricted peptides, selected on the basis of Treg cell epitope sequences (Tregitopes) on the IgG Fc and F(ab)2 regions, suppress immune responses to coadministered antigens by stimulating the expansion of Treg [47]. Combined allergen with adjuvant exposure by intranasal treatment with OVA encased oligomannose-coated liposomes was also found to expand Treg and suppressive CD8⁺ T-cells, while reducing OVA-specific IgE and allergic symptoms in mice by targeting specific ICAM-3 grabbing non-integrin-related and complement receptor 3 on dendritic cells [48]. Biologic agents, such as *Lactobacillus rhamnoses* (Lcr35), may also install tolerogenic dendritic cells. Adoptive transfer of Lcr35-treated dendritic cells or feeding Lcr35 prior to sensitization lowered the allergic airway response in OVA-allergic mice, while enhancing Treg in the mesenteric lymph nodes [49].

DIETARY INTERVENTION

Dietary components favouring SIT may involve herbal formula-2 (FAHF-2), probiotic mixture VSL#3, nondigestible oligosaccharides or n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA) (Fig. 1). FAHF-2, a traditional Chinese medicine cocktail of nine herbs, of which active components include alkaloids berberine, palmatine and jatrorrhizine, successfully treated peanut allergy in mice, without using allergen. Beyond systemic T_H1 polarization FAHF-2 suppressed mast cell FcεRI expression and reduced mast cell and basophil numbers. FAHF-2 is currently being tested in a phase II trial in peanut-allergic patients [50]. Beyond using herbs also treatment with VSL#3, enhanced Treg while suppressing T_H2 and peanut-allergic symptoms in mice [51]. A 9:1 mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides (scGOS/lcFOS) reduces the incidence of atopic dermatitis in infants at risk [52]. In-vitro scGOS/lcFOS combined with bacterial CpG DNA enhanced release of soluble type lectin galectin-9

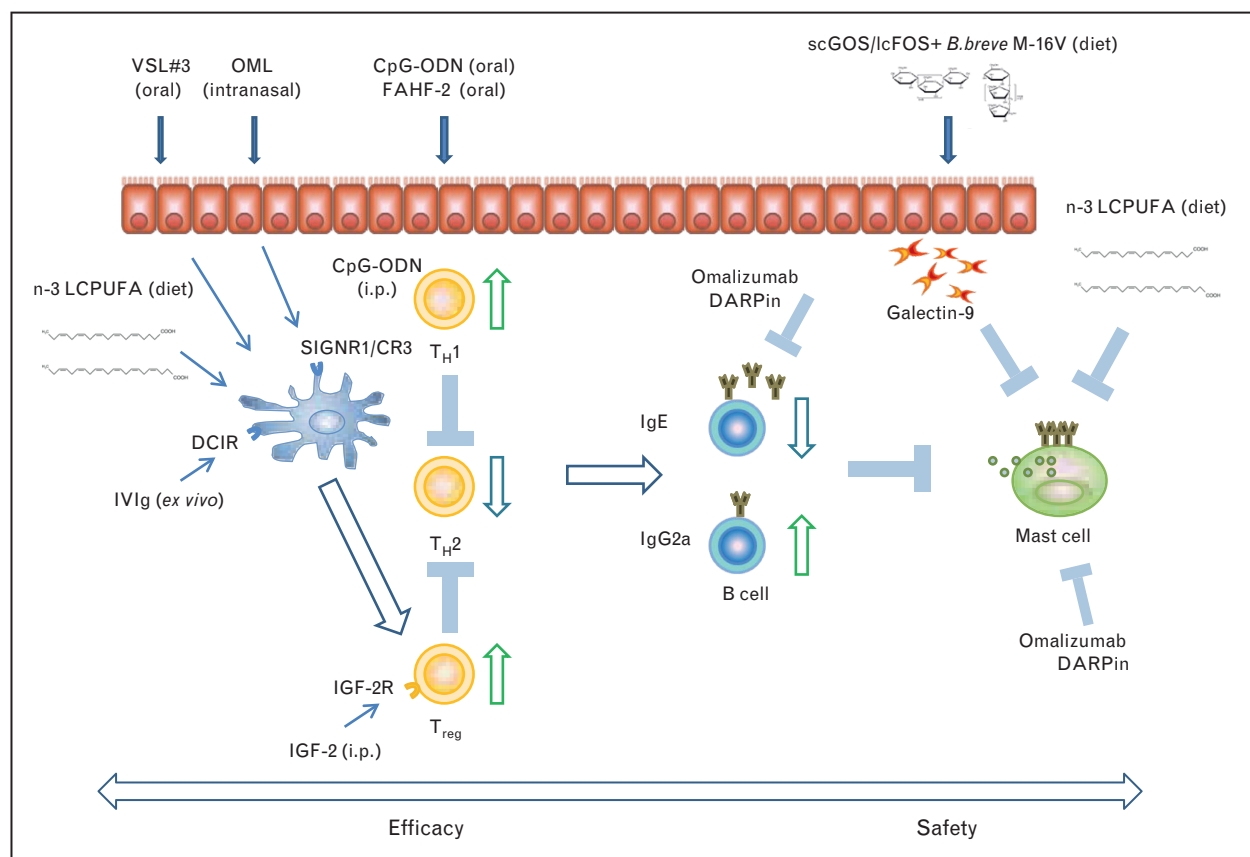


FIGURE 1. Future possibilities for adjunct therapy to be combined with SIT using adapted allergens or tolerogenic peptides in food allergy (preclinical data). CpG-ODN treatment enhanced systemic T_H1 (IFN- γ), reduced T_H2 (IL-13, IL-5) and decreased peanut IgE and/or increased IgG2a while suppressing mast cell degranulation and allergic symptoms in mice [41,42]. Similar findings were observed for FAHF-2 [50] and VSL#3 (a cocktail of four different lactobacilli spp., three bifidobacteria spp. and *Streptococcus thermophilus*) [51]. IGF-2 enhanced antigen-specific Treg by binding IGF-2R on Treg and suppressed T_H2 (IL-4, IL-13, IL-5), OVA-specific IgE, and allergic symptoms, when injected during oral OVA sensitization of mice [7^{***}]. IVIg binds DCIR hereby inducing tolerogenic DC that instruct Treg and prevent OVA induced airway hyperresponsiveness [46^{***}]. OVA encased oligomannose-coated liposomes instruct tolerogenic DC via SIGNR1 and CR3 and intranasal treatment suppressed food-allergic symptoms in OVA-sensitized mice [48]. Omalizumab and DARPin bind IgE and induce internalization of Fc ϵ R1 contributing to desensitization [39]. Dietary scGOS/lcFOS and *Bifidobacterium breve* M-16V increase galectin-9, and reduce allergic symptoms when provided during whey sensitization [54]. Dietary n-3 LCPUFA enhanced the frequency of tolerogenic DC and Treg and prevented allergic sensitization and symptoms in mice [55]. CpG-ODN, CpG-oligodeoxynucleotides; CR3, complement receptor 3; DARPin, designed ankyrin repeat proteins; DC, dendritic cells; IGF-2, insulin-like growth factor-2; LCPUFA, long-chain polyunsaturated fatty acids; OVA, ovalbumin; SIGNR1, specific ICAM-3 grabbing nonintegrin-related 1; SIT, specific immunotherapy; scGOS/lcFOS, short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides; Treg, regulatory T-cells.

(known to bind IgE) by human intestinal epithelial cells and instructed Treg and T_H1 polarization [53]. Dietary intervention with scGOS/lcFOS reduced allergic symptoms in whey-sensitized mice in correlation with increased serum galectin-9 levels, most effectively when combined with *Bifidobacterium breve* M-16V [54]. Furthermore, n-3 LCPUFA, found in oily fish, prevented allergic sensitization in mice by increasing the frequency of tolerogenic dendritic cells and Treg and suppressing the allergic effector

response [55,56]. Studies are warranted using these dietary components as adjunct therapy in SIT.

CONCLUSION

Improvement of SIT regarding persistence of clinical tolerance while reducing risk of treatment-related side-effects is needed. Safety of SIT can be enhanced by using modified allergens. Heat treatment retains desensitizing properties of allergens, while reducing

allergenicity, but is not tolerated by all patients. Small peptides derived from immunodominant T-cell epitopes of allergens, ultimately matching the HLA background of the patient, are now being tested for their tolerogenic properties. Beyond allergen modification, adjunct therapies are developed. Combining Omalizumab with OIT may improve safety, and future adjunct therapies for safety may involve DARPins, which bind IgE and effectively dissociate IgE from FcεRI. SIT efficacy can be enhanced by instructing T_H1 polarization and combined exposure of CpG-ODN with modified allergens, for example, packaged in nanoparticles, could facilitate this. Preclinical studies have shown IGF-2, sialic acid IVI9, Tregitopes or allergen encased oligomannose-coated liposomes capable of inducing allergen-specific Treg, which may be key for clinical tolerance induction and maintenance. Dietary FAHF-2 induces T_H1 polarization and suppresses effector responses, and is now tested in a phase II clinical trial in peanut-allergic patients. VSL#3 induces Treg, and TGF-β depending T_H2 suppression while lowering peanut-allergic symptoms in mice. Furthermore, dietary scGOS/lcFOS plus *B. breve* M-16V enhanced serum galectin-9 levels correlating with reduced allergic symptoms in mice and may be explored as SIT adjunct therapy. Furthermore, adequate n-3 LCPUFA status may facilitate a favourable milieu for tolerance induction. Combining SIT using (adapted) allergens or tolerogenic peptides with adjunct therapy may be essential to further improve safety and/or efficacy.

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Conflicts of interest

Authors do not declare a conflict of interest. Professor dr. Johan Garssen is employed at Nutricia Research B.V. and the Utrecht University. Dr Leon M.J. Knippels is employed at Nutricia Research B.V.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Prescott SL. Early-life environmental determinants of allergic diseases and the wider pandemic of inflammatory noncommunicable diseases. *J Allergy Clin Immunol* 2013; 131:23–30.
2. Moran TP, Vickery BP, Burks AW. Oral and sublingual immunotherapy for food allergy: current progress and future directions. *Curr Opin Immunol* 2013; 25:781–787.
3. Kostadinova AI, Willemsen LE, Knippels LM, *et al.* Immunotherapy - risk/benefit in food allergy. *Pediatr Allergy Immunol* 2013; 24:633–644.
4. Oka T, Rios EJ, Tsai M, *et al.* Rapid desensitization induces internalization of antigen-specific IgE on mouse mast cells. *J Allergy Clin Immunol* 2013; 132:922.e1–932.e16.
5. Bartnikas LM, Gurish MF, Burton OT, *et al.* Epicutaneous sensitization results in IgE-dependent intestinal mast cell expansion and food-induced anaphylaxis. *J Allergy Clin Immunol* 2013; 131:451.e1–460.e6.
6. Syed A, Garcia MA, Lyu SC, *et al.* Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3). *J Allergy Clin Immunol* 2014; 133:500–510.
- In this study, successful peanut OIT resulted in increased functional allergen induced Treg that were high in FoxP3 expression, and reduced methylation of the FoxP3 locus was associated with clinical tolerance induction.
7. Yang G, Geng XR, Song JP, *et al.* Insulin-like growth factor 2 enhances regulatory T-cell functions and suppresses food allergy in an experimental model. *J Allergy Clin Immunol* 2014; 133:1702.e5–1708.e5.
- This study shows selective insulin-like growth factor receptor expression on human and murine Treg. IGF-2 induces Treg proliferation and suppressive function upon combined TCR signalling.
8. Nurmatov U, Devereux G, Worth A, *et al.* Effectiveness and safety of orally administered immunotherapy for food allergies: a systematic review and meta-analysis. *Br J Nutr* 2014; 111:12–22.
9. Keet CA, Seopaul S, Knorr S, *et al.* Long-term follow-up of oral immunotherapy for cow's milk allergy. *J Allergy Clin Immunol* 2013; 132:737.e6–739.e6.
- Follow-up of two controlled trials of OIT for cow's milk allergy indicates that over time previously obtained desensitization is lost in a large percentage of patients.
10. Anagnostou K, Islam S, King Y, *et al.* Assessing the efficacy of oral immunotherapy for the desensitisation of peanut allergy in children (STOP II): a phase 2 randomised controlled trial. *Lancet* 2014; 383:1297–1304.
11. Blumchen K, Beder A, Beschoner J, *et al.* Modified oral food challenge used with sensitization biomarkers provides more real-life clinical thresholds for peanut allergy. *J Allergy Clin Immunol* 2014; 134:390–398.
12. Chin SJ, Vickery BP, Kulis MD, *et al.* Sublingual versus oral immunotherapy for peanut-allergic children: a retrospective comparison. *J Allergy Clin Immunol* 2013; 132:476.e2–478.e2.
- Patients undergoing OIT were three times more likely to pass double-blind placebo-controlled food challenge at 12 months compared with SLIT. However, the safety profile of SLIT was better because of less adverse effects.
13. Vickery BP, Lin J, Kulis M, *et al.* Peanut oral immunotherapy modifies IgE and IgG4 responses to major peanut allergens. *J Allergy Clin Immunol* 2013; 131:128.e1–134.e3.
14. Vickery BP, Scurlock AM, Kulis M, *et al.* Sustained unresponsiveness to peanut in subjects who have completed peanut oral immunotherapy. *J Allergy Clin Immunol* 2014; 133:468.e6–475.e6.
15. Meglio P, Giampietro PG, Carello R, *et al.* Oral food desensitization in children with IgE-mediated hen's egg allergy: a new protocol with raw hen's egg. *Pediatr Allergy Immunol* 2013; 24:75–83.
16. Dello Iacono I, Tripodi S, Calvani M, *et al.* Specific oral tolerance induction with raw hen's egg in children with very severe egg allergy: a randomized controlled trial. *Pediatr Allergy Immunol* 2013; 24:66–74.
17. Badina L, Matarazzo L, Longo G, *et al.* Could slightly cooked egg be a suitable medium for oral immunotherapy in persistent hen's egg allergy? *Allergol Immunopathol (Madr)* 2013; 41:141–142.
18. Fleischer DM, Burks AW, Vickery BP, *et al.* Sublingual immunotherapy for peanut allergy: a randomized, double-blind, placebo-controlled multicenter trial. *J Allergy Clin Immunol* 2013; 131:119.e1–127.e7.
- This randomized double-blind placebo-controlled multicenter trial obtained desensitization of 70% in SLIT patients for peanut allergy and indicated its general safety.
19. Jones SM, Burks AW, Dupont C. State of the art on food allergen immunotherapy: oral, sublingual, and epicutaneous. *J Allergy Clin Immunol* 2014; 133:318–323.
20. Dioszeghy V, Mondoulet L, Dhelft V, *et al.* The regulatory T cells induction by epicutaneous immunotherapy is sustained and mediates long-term protection from eosinophilic disorders in peanut-sensitized mice. *Clin Exp Allergy* 2014; 44:867–881.
21. Feldman MF, Bird JA. Oral immunotherapy for food allergy, ready for prime time? Heated egg and milk. *Curr Allergy Asthma Rep* 2014; 14:436.
22. Turner PJ, Mehr S, Joshi P, *et al.* Safety of food challenges to extensively heated egg in egg-allergic children: a prospective cohort study. *Pediatr Allergy Immunol* 2013; 24:450–455.
23. Watanabe H, Toda M, Sekido H, *et al.* Heat treatment of egg white controls allergic symptoms and induces oral tolerance to ovalbumin in a murine model of food allergy. *Mol Nutr Food Res* 2014; 58:394–404.
24. Leonard SA, Martos G, Wang W, *et al.* Oral immunotherapy induces local protective mechanisms in the gastrointestinal mucosa. *J Allergy Clin Immunol* 2012; 129:1579.e1–1587.e1.
25. Leonard SA, Sampson HA, Sicherer SH, *et al.* Dietary baked egg accelerates resolution of egg allergy in children. *J Allergy Clin Immunol* 2012; 130:473.e1–480.e1.
26. Gupta K, Kumar S, Das M, *et al.* Peptide based immunotherapy: a pivotal tool for allergy treatment. *Int Immunopharmacol* 2014; 19:391–398.

27. Rupa P, Mine Y. Oral immunotherapy with immunodominant T-cell epitope peptides alleviates allergic reactions in a Balb/c mouse model of egg allergy. *Allergy* 2012; 67:74–82.
28. Prickett SR, Voskamp AL, Dacumos-Hill A, *et al.* Ara h 2 peptides containing dominant CD4+ T-cell epitopes: candidates for a peanut allergy therapeutic. *J Allergy Clin Immunol* 2011; 127:608.e1–615.e5.
29. Prickett SR, Voskamp AL, Phan T, *et al.* Ara h 1 CD4+ T cell epitope-based peptides: candidates for a peanut allergy therapeutic. *Clin Exp Allergy* 2013; 43:684–697.
30. Bonnelykke K, Matheson MC, Pers TH, *et al.* Meta-analysis of genome-wide association studies identifies ten loci influencing allergic sensitization. *Nat Genet* 2013; 45:902–906.
- Ten susceptibility loci were defined in the first large-scale genome-wide association study on allergic sensitization, including single nucleotide polymorphisms in HLA-B and -DOB1.
31. Lanzavecchia A, Bove S. Specific B lymphocytes efficiently pick up, process and present antigen to T cells. *Behring Inst Mitt* 1985; 77:82–87.
32. Pascal M, Konstantinou GN, Masilamani M, *et al.* In silico prediction of Ara h 2 T cell epitopes in peanut-allergic children. *Clin Exp Allergy* 2013; 43:116–127.
33. Meulenbroek LA, van Esch BC, Hofman GA, *et al.* Oral treatment with beta-lactoglobulin peptides prevents clinical symptoms in a mouse model for cow's milk allergy. *Pediatr Allergy Immunol* 2013; 24:656–664.
- This study identified peptides from bovine beta-lactoglobulin comprising T-cell epitopes and reported that oral preexposure to these peptides prevents allergic symptoms in a mouse model for cow's milk allergy.
34. Meulenbroek LA, den Hartog Jager CF, Levens AF, *et al.* Characterization of T cell epitopes in bovine alpha-lactalbumin. *Int Arch Allergy Immunol* 2014; 163:292–296.
35. Wang J, Sampson HA. Treatments for food allergy: how close are we? *Immunol Res* 2012; 54:83–94.
36. Zhou Y, Kawasaki H, Hsu SC, *et al.* Oral tolerance to food-induced systemic anaphylaxis mediated by the C-type lectin SIGNR1. *Nat Med* 2010; 16:1128–1133.
37. Wood RA, Sicherer SH, Burks AW, *et al.* A phase 1 study of heat/phenol-killed, E. coli-encapsulated, recombinant modified peanut proteins Ara h 1, Ara h 2, and Ara h 3 (EMP-123) for the treatment of peanut allergy. *Allergy* 2013; 68:803–808.
38. Ren C, Zhang Q, Wang G, *et al.* Modulation of peanut-induced allergic immune responses by oral lactic acid bacteria-based vaccines in mice. *Appl Microbiol Biotechnol* 2014; 98:6353–6364.
39. Eggel A, Baravalle G, Hobi G, *et al.* Accelerated dissociation of IgE-FcεRI complexes by disruptive inhibitors actively desensitizes allergic effector cells. *J Allergy Clin Immunol* 2014; 133:1709.e8–1719.e8.
40. Schneider LC, Rachid R, LeBovidge J, *et al.* A pilot study of omalizumab to facilitate rapid oral desensitization in high-risk peanut-allergic patients. *J Allergy Clin Immunol* 2013; 132:1368–1374.
41. Zhu FG, Kandimalla ER, Yu D, *et al.* Oral administration of a synthetic agonist of Toll-like receptor 9 potentially modulates peanut-induced allergy in mice. *J Allergy Clin Immunol* 2007; 120:631–637.
42. Kulis M, Gorentla B, Burks AW, *et al.* Type B CpG oligodeoxynucleotides induce Th1 responses to peanut antigens: modulation of sensitization and utility in a truncated immunotherapy regimen in mice. *Mol Nutr Food Res* 2013; 57:906–915.
43. Senti G, Johansen P, Haug S, *et al.* Use of A-type CpG oligodeoxynucleotides as an adjuvant in allergen-specific immunotherapy in humans: a phase I/IIa clinical trial. *Clin Exp Allergy* 2009; 39:562–570.
44. Beeh KM, Kannies F, Wagner F, *et al.* The novel TLR-9 agonist QbG10 shows clinical efficacy in persistent allergic asthma. *J Allergy Clin Immunol* 2013; 131:866–874.
45. Pali-Scholl I, Szollosi H, Starkl P, *et al.* Protamine nanoparticles with CpG-oligodeoxynucleotide prevent an allergen-induced Th2-response in BALB/c mice. *Eur J Pharm Biopharm* 2013; 85:656–664.
46. Massoud AH, Yona M, Xue D, *et al.* Dendritic cell immunoreceptor: a novel ■ receptor for intravenous immunoglobulin mediates induction of regulatory T cells. *J Allergy Clin Immunol* 2014; 133:853.e5–863.e5.
- Sialylated IVIg instructs tolerogenic dendritic cells via binding of the dendritic cell immunoreceptor and generates pulmonary Treg protecting mice against asthmatic symptoms.
47. Cousens LP, Najafian N, Mingozzi F, *et al.* In vitro and in vivo studies of IgG-derived Treg epitopes (Tregitopes): a promising new tool for tolerance induction and treatment of autoimmunity. *J Clin Immunol* 2013; 33 (Suppl 1): S43–S49.
48. Kawakita A, Shirasaki H, Yasutomi M, *et al.* Immunotherapy with oligomannose-coated liposomes ameliorates allergic symptoms in a murine food allergy model. *Allergy* 2012; 67:371–379.
49. Kim HJ, Kim YJ, Lee SH, *et al.* Effects of *Lactobacillus rhamnosus* on asthma with an adoptive transfer of dendritic cells in mice. *J Appl Microbiol* 2013; 115:872–879.
50. Wang J. Treatment of food anaphylaxis with traditional Chinese herbal remedies: from mouse model to human clinical trials. *Curr Opin Allergy Clin Immunol* 2013; 13:386–391.
51. Barletta B, Rossi G, Schiavi E, *et al.* Probiotic VSL#3-induced TGF-β ameliorates food allergy inflammation in a mouse model of peanut sensitization through the induction of regulatory T cells in the gut mucosa. *Mol Nutr Food Res* 2013; 57:2233–2244.
52. Moro G, Arslanoglu S, Stahl B, *et al.* A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. *Arch Dis Child* 2006; 91:814–819.
53. de Kivit S, Kraneveld AD, Knippels LM, *et al.* Intestinal epithelium-derived galectin-9 is involved in the immunomodulating effects of nondigestible oligosaccharides. *J Innate Immun* 2013; 5:625–638.
54. de Kivit S, Saeland E, Kraneveld AD, *et al.* Galectin-9 induced by dietary synbiotics is involved in suppression of allergic symptoms in mice and humans. *Allergy* 2012; 67:343–352.
55. van den Elsen LW, van Esch BC, Hofman GA, *et al.* Dietary long chain n-3 polyunsaturated fatty acids prevent allergic sensitization to cow's milk protein in mice. *Clin Exp Allergy* 2013; 43:798–810.
56. van den Elsen LW, Meulenbroek LA, van Esch BC, *et al.* CD25+ regulatory T cells transfer n-3 long chain polyunsaturated fatty acids-induced tolerance in mice allergic to cow's milk protein. *Allergy* 2013; 68:1562–1570.