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Animal models of allergen-specific immunotherapy in food allergy: Overview and opportunities

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Abbreviations
APC: Antigen Presenting Cell
CMP: Cow's milk protein
CT: Cholera toxin
dNL: Draining lymph node
EP: Epicutaneous
EPIT: Epicutaneous Immunotherapy
HK: Heat-killed
ID: Intradermal
IG: Intragastric
IN: Intranasal
IP: Intraperitoneal
IPIT: Intraperitoneal Immunotherapy
IT: Immunotherapy
IV: Intravenous
mLN: Mesenteric lymph node
PR: Per rectum
SC: Subcutaneous

SCIT: Subcutaneous immunotherapy
SLIT: Sublingual IT
Treg: Regulatory T cell

Abstract

Food allergy is an adverse reaction to otherwise harmless proteins in food. The disease is a major health problem of growing concern, affecting approximately 5-8% of young children and 2-4% of adults. No accepted strategy exists for prevention and treatment of food allergy, and strict avoidance of the offending food is presently the only viable management option. Living with food avoidance may have a huge impact on the quality of life of food allergic patients, with daily fear of serious or even fatal reactions. The urgent need for safe and efficient food allergy treatment options has led to massive research efforts to develop and improve strategies for food allergy immunotherapeutic approaches. A first step in developing new and improved strategies of immunotherapy often involves the use of animal models. In present review, we provide an overview of animal studies of allergen-specific immunotherapy highlighting opportunities and challenges for each approach. The presented models, almost exclusively performed in mice, assess therapeutic efficacy and immunological outcomes following oral, intraperitoneal, subcutaneous, epicutaneous, and sublingual administration of native allergens, or preparations of hydrolyzed allergen, T cell directed peptides, or allergen with immunomodulatory adjuvants. Recently, approaches using immune cell therapy have demonstrated efficacy. Current models mainly assess anaphylaxis as the primary clinical outcome. With the increased appreciation that food allergy is a heterogeneous disease presenting different phenotypes, there is a continued need to develop new disease-relevant therapeutic models of food allergy.

Introduction

Food allergy is an immune mediated hypersensitivity to specific proteins in food, which occur as a result of allergic sensitization due to a failure of the immune system to develop tolerance after first exposure, or the abrogation of an already established tolerance. The prevalence

of food allergy is estimated to be around 5-8% in young children and around 2-4% in adults in the Western world [1], and appears to be a rising problem [1,2]. Most cases of food allergy is mediated by IgE-dependent immediate hypersensitivity reactions, however some rarer food allergies may also involve cell-mediated sensitization with and without the contribution of IgE (extensively reviewed in [3,4]). Ingestion of disease-triggering foods in sensitized individuals can cause an array of symptoms in the gastrointestinal tract (pain, nausea, vomiting and diarrhea), skin (itchiness, urticarial and angioedema), respiratory and cardiovascular systems (dyspnea, wheezing, and tachycardia) [5]. In severe cases allergic reactions can cause acute respiratory and cardiac arrest. The first line of management involves food-avoidance, and acute systemic reactions are treated with epinephrine, which are often carried by patients with severe allergy in case of accidental ingestion. The lack of treatment options, restrictive food-avoidance, and the prospect of accidental ingestion makes food allergy a chronic disease that significantly impacts the quality-of-life for patients [6].

Allergen-specific immunotherapy (IT) is emerging as a viable option for human desensitization with the ability to increase thresholds of reactivity, as demonstrate by a recent systematic meta-analysis of 31 clinical trials in food allergy [7]. However, current immunotherapeutic regimens are typically lengthy with repeated, increasing doses of allergen, and the risk of acute adverse reactions. Furthermore, current regimens are largely unable to induce complete clinical tolerance. Thus there is a need to develop better regimens of allergen-specific IT. Testing therapies in animal disease models is central to evaluating the potential of new immunotherapeutic strategies or modified versions of current regimens. Here we present a comprehensive overview of animal models of food allergy therapy currently reported in the literature, and highlight opportunities for new avenues of study and model improvements. This review will primarily address IT in animal models using allergens and allergen-challenges of relevance to clinical food allergy outcomes. A detailed overview of the presented therapeutic animal models can be found in table 1, and summarized in figure 1. Currently most models have been developed in mice exploring oral therapy

with dosaging of native protein, and to some extent less allergenic preparations containing hydrolyzed allergen and immunodominant T cells epitopes. Models using epicutaneous (EPIT) or subcutaneous immunotherapy (SCIT) has in recent years received increased attention due to possible better safety and efficacy of these routes compared to oral administration. Additionally, models exploring therapy using immunomodulatory adjuvants or cell therapy have been developed. Current studies have addressed several immunological and clinical outcomes of therapy (summarized in figure 2). However, most models only assess the acute anaphylaxis outcome of food allergy by temperature loss and clinical severity scoring. With the increased appreciation that food allergy is a heterogeneous disease presenting with different phenotypes and mechanisms in relation to foods, exposure, symptoms, and natural history [8], there is a continued need to develop new disease-relevant therapeutic models in food allergy.

Oral immunotherapy

The default outcome of ingesting food is for the immune system to tolerate the exposure to the foreign antigens contained within. The concept of oral tolerance has been studied in rodents since 1910, with early studies describing how oral ingestion of antigen would inhibit later experimental hyperactivity, thus demonstrating that tolerance to ingested food involves active antigen-specific suppression of hypersensitivity [9]. Several later studies have addressed the mechanistic basis of oral tolerance, including a pivotal role of regulatory T cells (reviewed in [10,11]). Oral IT (OIT) models have been developed using egg white [12], ovomucoid (native or heated) [12], ovalbumin [13], cow's milk proteins [14,15] and peanut [15–17] in sensitized mice. In these studies therapy were able to reverse or ameliorate food challenge-induced anaphylaxis and clinical disease scores, when mice were challenged immediately after treatment. However some studies found [12,13,17], that the desensitization was short-term as sensitization returned within 2-5 weeks after therapeutic cessation. These findings are in line with oral immunotherapy trials in humans reporting limited effects on the induction of sustained unresponsiveness in food allergic individuals. The OIT

models found varying effect on the levels of allergen-specific IgG reporting similar [12–14], decreased [17], or increased [15] IgG1 levels, and similar [12–14] or increased [13,15,16] IgG2a levels. The therapeutic effect on allergen-specific IgE levels were similarly diverse with similar [13,15], increased [12] or decreased [14,16,17] levels reported. These findings could be attributed to the dynamic nature of Ig levels during IT, and are in line with studies in humans reporting different Ig patterns depended on IT efficacy and duration [18,19]. Interestingly, the studies in ovalbumin-sensitized mice observed sustained systemic allergen-specific humoral immune responses after therapy, as demonstrated by basophil activation assays and peritoneal mast cells activation [12,13]. Thus the mice was shown to be desensitized when orally challenged with allergen, but anaphylaxis could be induced by systemic allergen-challenge [12]. Similar results were observed in another study where peanut and whey OIT suppressed mast cell responses following oral challenge, but only mice receiving peanut OIT were protected from intraperitoneal (IP) induced anaphylaxis, indicating allergen-specific differences in therapeutic outcomes and mechanisms [15]. The therapeutic suppression of local gastrointestinal allergic responses was proposed to be mediated by increased IgA levels induced by treatment; however this hypothesis remains to be formally investigated [12]. Oral therapy was also associated with increased gastrointestinal permeability and changes in transcriptional profile in proximal jejunum [12]. The significance of these treatment-induced changes remain to be investigated, but may reflect the adverse gastrointestinal effects reported in human clinical trials.

Allergen-specific *ex vivo* production of T cell related type-2 cytokines (IL-4, IL-5 and/or IL-13) were suppressed after egg [12], cow's milk protein [14,15] and peanut [16] OIT, indicating suppression of systemic effector T cell immune responses. Indeed the studies found increased numbers of $CD4^+CD25^+FoxP3^+$ regulatory T cells in the intestine or mLN in response to milk protein [14,15] or peanut [16] therapy. The induction of intestinal regulatory T cells was associated with increased tissue expression of regulatory effector cytokines TGF-β and IL-10 [14]. Unexpectedly, IT in

ovalbumin-sensitized mice did not result in the generation of CD4$^+$CD25$^+$FoxP3$^+$ regulatory T cells [13], and *ex vivo* allergen-specific IL-10 was suppressed in mice receiving OIT [12]. These results suggest the induction of different immune regulatory mechanisms in the therapeutic response to different allergens or regimens. The study using peanut IT [16] found, that the expansion of regulatory T cells was abrogated eight weeks post-therapy, and the induced regulatory T cells were unable to transfer desensitization to sensitized mice. These findings suggest that the short-term desensitization in these models is due to the unstable induction of regulatory T cells. Interestingly, a study combining peanut OIT and abrogation of IgE signaling by anti-IgE antibody or Syk inhibitor administration in allergy-prone Il4raF709 (gain-of-function mutation in the IL-4 receptor α chain) mice found induction of sustained unresponsiveness associated with expansion of regulatory T cells and suppression of allergen-specific Th2 cells [17]. These findings are in line with recent studies in humans, where anti-IgE can facilitate accelerated desensitization by oral dosing of allergen [20]. Combined, the studies presented here suggest that induction of allergen-specific regulatory T cells is pivotal for efficient IT of food allergy, and different allergens and therapeutic regimens may induce regulatory T cells with different phenotypes. There is a need to study co-administration of immune modulatory reagents (TLR agonists and biologicals) in OIT models, and some examples are discussed later in the review.

Intraperitoneal immunotherapy

The studies presented above performed IT to food proteins via the oral route, however a couple of studies have used intraperitoneal (IP) injections with tree nut (cashew and walnut) protein extracts as a model of oral therapy [21,22]. Cashew intraperitoneal IT (IPIT) was able to abrogate allergic reactions in response to cashew [21] or pistachio [22] (due to cross-reactivity) in cashew-sensitized mice. Furthermore, cashew-, walnut- or combined-IT was able to ameliorate anaphylaxis (temperature loss) and clinical disease presentation in cashew and walnut dual-sensitized mice due to cross-reactivity between these three nuts [22]. IT was associated with

increased allergen-specific IgG1 [21,22], IgG2 [21] and suppression of T cell related type-2 cytokine release (IL-4, IL-5 and IL-13) [21,22] by splenocytes. Interestingly, in these models challenges were performed IP 2-5 weeks post-treatment indicating long-term efficacy of therapy. These findings are opposed to the studies of OIT reviewed above, in which efficacy to oral challenges was lost 2-4 weeks post-treatment and allergic reactivity was sustained in response to IP (systemic) challenge for ovalbumin and whey OIT [12,15]. This indicates that the therapeutic tolerance induced via the oral route is different from tolerance generated via IP allergen administration. It could be speculated that oral allergen administration subverts long-term protection by chronic activation of IgE-dependent mechanisms, as indicated in the case of oral peanut desensitization in combination with anti-IgE [17]. However, more experimental work comparing administration routes using the same allergens, sensitization and challenge protocols are required to address this hypothesis. Furthermore, the role of regulatory T cells in IPIT models remains to be investigated.

Immunotherapy using hydrolyzed food proteins

It is generally accepted, that hydrolyzed food allergens exhibit lower allergenicity due the destruction of allergenic epitopes. It has been demonstrated, that pepsin-digested cashew protein extracts elicit a reduced anaphylactic response (temperature loss and symptom score) compared to undigested protein in a murine food allergy model using IP or oral sensitization to undigested cashew protein [21]. Both digested and undigested cashew protein were show to have similar allergenicity by inducing comparable IgE- and Th2-responses, and clinical anaphylaxis following challenge in mice sensitized to the respective preparations using adjuvant. These observations demonstrate the principle, that IT using digested protein has reduced potential to elicit an allergic response during therapy to native food protein. The study continued to use digested cashew protein as IPIT in mice sensitized to undigested cashew protein [21]. IT was demonstrated to abrogate anaphylaxis when the mice were challenged with undigested protein. Furthermore, therapy was associated with the induction of allergen-specific IgG1 and IgG2a, as well as suppression

of IL-5 and IL-13 production by splenocytes. The clinical and immunological outcomes were comparable to mice receiving IPIT using undigested protein extract, indicating the same mechanism of desensitization. OIT using hydrolyzed egg white proteins in egg white sensitized mice was indicated to ameliorate anaphylaxis as demonstrated by normalizing serum histamine levels following challenge [23]. Therapy was associated with a modest reduction in allergen-specific IgE and IgG2a, whereas IgG1 was unchanged. Allergen-stimulated splenocytes demonstrated reduced systemic Th1 (IFN-γ) and Th2 (IL-4) responses. Furthermore, expression of Th1 and Th2 effector cytokines (IFN-γ, IL-12p40, IL-4 and IL-13) was reduced in intestinal tissue; however IL-18 and IL-5 were increased. Therapy was found to promote expression of immune regulatory genes TGF-β and FoxP3 but suppress IL-10 in intestinal tissue, suggesting the generation of regulatory T cells in therapy using digested allergens. As discuss in previous sections, IPIT and OIT may elicit different mode of desensitization, and oral therapy may give rise to specific gut protection and adverse reactions depended on the allergen [12,15]. Thus, OIT using digested and undigested protein needs to be compared head-to-head to determine altered efficacy, reduced adverse effects and mechanism of desensitization.

T cell directed peptide immunotherapy

IT using specific peptides may be a more specific alternative to the use of digested or hydrolyzed food proteins allowing a consistent and well-defined therapy. Several studies have systematically assayed specific peptides from allergens to identify T cell epitopes by the means of stimulating immune cells from sensitized animals or allergic patients followed by measuring proliferation or cytokine production. Allergen immunodominant T cell epitopes have been identified for several food allergens for mice (ovalbumin [24], ovomucoid [25], beta-lactoglobulin [26,27], and shrimp tropomyosin [28]) and human (peanut Ara h 1 [29,30] and Ara h 2 [31,32], and shrimp tropomyosin [33]). Oral administration of murine immunodominat peptides have been shown to ameliorate or reverse anaphylaxis, disease scores and/or diarrhea in mice sensitized to ovalbumin

[34], ovomucoid [35], shrimp tropomyosin [28], and beta-lactoglobulin [36]. The studies of ovomucoid and beta-lactoglobulin reported a therapeutic effect of single peptide administration (15- and 22-mers) [35,36], whereas none of three different peptides (15-mers) in the ovalbumin study had an effect when administered individually [34]. However, administration of the three ovalbumin peptides as a cocktail significantly ameliorated clinical anaphylactic scores and systemic histamine levels, suggesting varying efficacy of different T cell epitopes. The study in shrimp-tropomyosin sensitized mice compared a cocktail of six immunodominant and six non-immunodominat peptides (20-mers) demonstrating specific efficacy of immunodominant peptide IT [28]. Therapy directed against ovalbumin [34], ovomucoid [35] or shrimp-tropomyosin [28] reported decreased allergen-specific IgE levels and suppression of type-2 immune responses (IL-4, IL-5 and IL-13) in the intestine or following *ex vivo* stimulation. Additionally, these studies found therapeutic induction of immune regulatory mechanisms indicated by increased intestinal expression of FoxP3, TGF-β and/or IL-10 [28,34], as well as increased allergen-specific IL-10 production and systemic $CD4^+FoxP3^+$ regulatory T cell numbers [35]. The study using peptide IT in beta-lactoglobulin sensitized mice was unable to report therapeutic effects on antibody levels (allergen-specific IgE, IgG1, IgG2a, IgA, or total IgE), allergen-specific immune responses *ex vivo* (IL-10, IFN-γ, IL-10, and IL-12), or splenic $CD4^+CD25^+FoxP3^+$ regulatory T cell numbers, suggesting different mode of action or experimental difficulties in this study [36].

OIT using immunodominant peptides show therapeutic potential, but long-term efficacy remains to be studied in these pre-clinical models. Unfortunately, recent clinical trials using Fel d 1 peptide therapy in humans have been disappointing in terms of therapeutic effect (Circassia Pharmaceuticals plc, June 20th 2016 press release, www.circassia.com). This may be attributed to the need for immunodominant peptides that could be different among patients due to genetic HLA diversity. Indeed, systematic screening reveal different peptide epitopes among different mouse stains for ovomucoid [25] and beta-lactoglobulin [26,27].

Subcutaneous immunotherapy

Administrating IT via other routes than oral may elicit different clinical and immunological outcomes when the food allergy expressing organ is not targeted directly. Subcutaneous (SC) injections have for decades been used in IT of allergic disease with demonstrated efficacy in asthma, rhinitis, and venom allergies [37]. However, the use of subcutaneous IT (SCIT) in food allergy was likely abandoned in the 1990s due to high rates of adverse systemic reactions in peanut allergic patients [38,39]. A recent study compared SCIT and OIT in models of cow's milk (whey) and peanut allergy [15]. SCIT and OIT were able to suppress anaphylaxis and mast cell degranulation (serum mMCP-1) following intragastric (IG) or intradermal (ID) challenge in both models with no clear differences in route-dependent efficacy. Rather, clinical allergen-dependent differences were indicated by the observation that neither SCIT nor OIT could protect from IP induced anaphylaxis in whey sensitized mice, but peanut sensitized mice were protected in a therapeutic dose-dependent manner. Both SCIT and OIT increased serum allergen-specific IgG1 and IgG2a, whereas only SCIT increased allergen-specific IgE. This may be in line with the observation, that only OIT suppressed (whey) or had similar (peanut) allergen-specific type-2 cytokines production (IL-5 and IL-13) while SCIT was similar (whey) or increased (peanut). The authors addressed the frequency of $CD4^+CD69^+ST2^+$ Th2, $CD4^+CD69^+CXCR3^+$ Th1 and $CD4^+CD25^+FoxP3^+$ regulatory T cells in spleen or mLN, however no clear allergen- or therapy-related patterns could be observed suggesting diverse underlying immunological mechanisms [15]. Other SCIT mouse models using food allergens support efficacy, but have been conducted without oral or IP allergen provocations for the assessment of clinical food allergy outcomes. Studies using ovalbumin or peanut SCIT found therapy to suppress allergen-specific IgE and increase IgG2a in serum [40,41]. In line, SCIT treated animals exhibited decreased airway hyperreactivity and suppressed type-2 immune responses in the lung following airway allergen challenge. Additional mechanistic studies in SCIT models employing clinical food allergy-related outcomes are needed, including therapeutic duration, and adverse reactions compared to OIT.

Epicutaneous immunotherapy

Epicutaneus IT (EPIT) has recently emerged as a new mode of allergen delivery in food allergy therapy. It was hypothesized that applying allergen onto intact skin would allow controlled diffusion, and reduce risk of systemic allergen dissemination and anaphylaxis. To this end, DBV Technologies developed an epicutaneus delivery system (Viaskin patch) that consists of a chamber that releases allergen onto the skin [41], where it diffuses into the epidermis and is taken up by dendritic cells that migrates to draining lymph nodes and induce allergen-specific regulatory T cells [42]. Early studies found that EPIT could suppress allergen-specific IgE and type-2 immune responses, and increase allergen-specific IgG2a in ovalbumin [41,42], peanut [40,41], cow's milk protein [43], house dust mite [41] and pollen [41,44] sensitized mice. The first studies of EPIT in relation to food allergy outcomes were conducted in models of esophago-gastro-enteropathy following long-term allergen exposure in sensitized animals. EPIT was found to completely reverse esophageal and gastric eosinophilia, and reduce serum allergen-specific IgE in peanut sensitized mice [45,46] and pigs [47]. Abrogated eosinophilia was associated with reduced eotaxin (CCL11) in mice esophagus and pig splenocytes. Furthermore, EPIT suppressed systemic allergen-specific type-2 (IL-5 and IL-13) immune responses in the models. The studies in mice reported increased CD4$^+$CD25$^+$FoxP3$^+$ regulatory T cells expressing CTLA-4, but not PD-1 or IL-10 in the spleen following EPIT [46]. The EPIT-induced regulatory T cells were found to suppress esophageal eosinophilia, eotaxin and IL-5 expression when adoptively transferred into non-treated peanut-sensitized animals. The regulatory T cells exhibited a stable phenotype as cells adoptively transferred from mice 8 weeks post-treatment retained the suppression of allergen-induced esophageal eosinophilia and systemic type-2 (IL-5 and IL-13) immune responses. These findings are supported by a more recent study demonstrating long-term stability of EPIT induced, but not OIT or sublingual IT (SLIT) induced, regulatory T cells [16]. EPIT-induced regulatory T cells were found to specifically express CCR9, CLA, CCR6, CCR3 and CCR8, suggesting that these cells can migrate and suppress allergic responses in several peripheral tissues, including the gut and skin. Interestingly, transfer of EPIT induced

regulatory T cells was found to expand the endogenous regulatory T cell population in recipient mice [46], which may explain why EPIT to cow's milk proteins suppress later sensitization to peanut and house dust mite in mice [43].

A recent study reported the therapeutic involvement of EPIT-induced regulatory T cells in a model of acute ovalbumin-induced anaphylaxis [13]. In this study did EPIT, but not OIT, induce long-term (>5 weeks) protection against anaphylaxis following oral challenge in ovalbumin-sensitized mice. Interestingly, humoral allergen-specific responses were not affected by treatment as demonstrated by basophil activation test and passive sensitization of naïve animal. Rather, allergen-specific $CD4^+LAP^+FoxP3^-$ regulatory T cells expressing CCR9, CCR6 and CCR4 were found to be induced in response to EPIT, but not OIT. These cells were induced in skin-draining lymph nodes and migrated to mLN and gut lamina propria. Adoptive transfer and cytokine blocking experiments in passively sensitized mice indicated that EPIT-induced regulatory T cells suppress mast cell activation in a TGF-β dependent manner.

The findings in EPIT models demonstrate that EPIT-induced regulatory T cells have a stable and distinct phenotype compared to regulatory T cells induced via other routes. Clinical trials in humans have shown that EPIT is safe, however the current strategies have demonstrated limited efficacy [48]. Further technical improvements of EPIT delivery, including skin preparation and delivery matrix, are open for optimization and mechanistic exploration in animal models of food allergy.

Sublingual immunotherapy

Sublingual IT (SLIT) involves administration of allergen under the tongue followed by swallowing. This therapeutic route is commonly used in the treatment of allergic rhinitis and asthma with demonstrated efficacy in humans [49]. Clinical trials of SLIT in food allergy has been conducted

in humans, but indicated reduced efficacy in the treatment of peanut and cow's milk allergy compared to OIT [50,51]. To our knowledge, only one therapeutic animal model using SLIT in relation to food allergy outcomes has been published. The study was published very recently demonstrating clinical efficacy of SLIT in cow's milk sensitized mice using low dosages of allergen (pg to ng range) [52]. Therapy was associated with increased IL-10 and TGF-β levels in jejunum indicating a role of regulatory T cells. This is supported by the study mentioned earlier comparing the function and phenotype of regulatory T cells induced by SLIT, EPIT and OIT in peanut sensitized mice [53]. No marked therapeutic difference was observed between allergen administration routes in relation to increased allergen-specific IgG2a in serum and suppression of allergen-specific type-2 (IL-5 and IL-13) cytokines. SLIT and OIT did however increase allergen-specific IL-10 production compared to EPIT, which in the SLIT group was essential for the suppression of allergen-specific IL-5 and IL-13 *ex vixo*. These findings indicate that SLIT induces IL-10 producing regulatory Tr1 cells in this peanut model. The study further indicated that the SLIT-induced regulatory Tr1 cells were unstable, as regulatory T cells adoptively transferred 8 weeks post treatment to sensitized animals had lost the ability to suppress allergic esophageal eosinophilia and allergen-specific immune responses. Development of new SLIT food allergy animal models may further our understanding of the immunological mechanism of SLIT in this disease, and allow optimization of administration protocols and allergen formulation.

Immunotherapy using immunomodulatory adjuvants

Adjuvants are commonly used in vaccines to elicit a desired immunological response. Therapeutic studies in food allergy models have explored the ability of adjuvants to skew the allergic type-2 immune response towards a type-1 response using TLR9 agonists [54–57] or heat-killed (HK) bacteria [58,59]. Two of the studies using TLR9 agonists were performed in mice sensitized to peanut using OIT [56] with whole peanut or peanut protein extract [54,55]. The studies addressing the role of HK bacterial adjuvants were performed using subcutaneous or per rectum (PR) IT using

recombinant Ara h 1-3 proteins with modified epitopes reducing IgE binding in peanut sensitized mice [58,59]. The studies reported similar results with suppression of oral challenge-induced anaphylaxis, reduced allergen-specific IgE and increased IgG2a in groups receiving adjuvant allergen-specific therapy. Allergen-specific type-2 (IL-4, IL-5 and IL-13) immune responses were suppressed, whereas IFN-γ was increased. Similar results was obtained in a IPIT study in peanut sensitized mice comparing the action of class A, B, and C CpG-ODN TLR9 agonists, where therapeutic effect was reported for class B and C CpG-ODNs indicating that B cells are a target of TLR9 agonism [56]. Two studies performed several oral challenges indicating therapeutic efficacy for up to 10-16 weeks post treatment [55,59]. Combined, these findings indicate that Th1-driving adjuvants promote allergen-specific Th1 cells with a stable phenotype. Another study using TLR9 agonist in intradermal IT in ovalbumin sensitized mice found limited effect on anaphylaxis scores and temperature loss, but indicated improved survival following challenge [57]. This finding may indicate allergen-specific differences, or reflect different treatment regimens. The role of regulatory T cells in these models remains largely unknown. One study using TLR9 agonist found no therapeutic effect on allergen-specific TGF-β levels [55], whereas one model using HK bacterial reported increased allergen-specific TGF-β [59]. As HK bacteria contain several ligands stimulating innate immunity, it cannot be excluded that agonists targeting other pathogen-associated molecular pattern receptors than TLR9 can be involved in promoting regulatory T cells. Thus there is an opportunity to study the role of other adjuvants in these models to identify alternatives to complex whole bacterial adjuvants contain several unknown components and antigens. Additionally, targeting the gut microbiota composition using probiotics or prebiotics may be an option to modulate local immune responses. Administration of a mixture of probiotic bacteria to shrimp tropomyosin sensitized mice has previously been found to suppress type-2 immune responses and challenge-induced anaphylaxis [60]. Thus, combining probiotics and allergen-specific IT may lead to improved efficacy.

A recent study employed a synthetic cetylpyridinium chloride and oil based nanoemulsion adjuvant with type-1/17 driving properties for nasal peanut IT in mice [61]. The adjuvant was found to promote suppression of challenge-induced anaphylaxis, allergen-specific IgE, and type-2 (IL-4, IL-5, IL-33) immune responses, while increasing IgG2a and IgG2b and regulatory T cells. The induction of therapeutic efficacy was partly dependent on IL-10 highlighting an underlying role of immunoregulatory mechanisms in this type of adjuvant. The importance of enhancing immunoregulatory mechanisms during allergen-specific IT is further supported by a recent study combining SLIT and the expansion of regulatory T cells using IL-2/anti-IL-2Ab complexes in cow's milk sensitized mice [52].

An interesting study used peanut protein chemically coupled to syngeneic splenic leukocytes as intravenous (IV) IT in peanut sensitized mice [62]. The chemical linking induces apoptosis in leukocytes making the cells a therapeutic vehicle and adjuvant in allergen-specific IT. Previous studies suggest that the apoptotic leukocytes are taken up by splenic marginal zone APCs leading to the induction of clonal anergy and regulatory T cell specific for the coupled antigen. IT in peanut sensitized mice was found to reverse challenge-induced blood eosinophilia and suppress allergen-specific type-2 (IL-4 and IL-13) responses, but had only a week effect on anaphylactic scores. This IT approach may need optimization in terms of chemical linking, cell subsets, and control of apoptosis/necrosis. Other vehicles for allergen could be explored for IV administration, including synthetic particles, coupling to APC-receptor specific targets, and allergen complexes.

Cellular immunotherapy

IT using immune cells has been used in the clinic to promote anti-cancer immunity, commonly by administrating *ex vivo* expanded effector T cells or dendritic cells carrying cancer antigens [63]. To our knowledge, cellular IT has not been applied in clinical allergy, which likely is reflected by the very few studies of this therapeutic approach in food allergy animal models. The

generation and transfer of allergen-specific regulatory T cells could be a potential therapy. This was recently explored in a model of peanut allergy where mice received regulatory T cells from mice having received OIT, SLIT or EPIT with peanut [16]. The study found that EPIT-induced regulatory T cells could suppress the elicitation of esophageal eosinophilia. Although the main objective was to assess the phenotypic stability of regulatory T cells induced by various routes of IT, this study provides a model of exploring this type of IT.

Dendritic cells play a central role in instructing T cell responses based on molecular signals from pathogens and other environmental stimuli. A recent study exploited that retinoic acid stimulates the development of a tolerogenic dendritic cell phenotype (RA-DCs). RA-DCs was generated *in vitro* and pulsed with ovalbumin or peanut protein before being administrated IP to sensitized mice [64]. RA-DC therapy suppressed challenge-induced anaphylaxis and diarrhea in both models. Therapeutic efficacy was dependent on IL-27 production by the RA-DCs, and *in vitro* experiments found that RA-DCs stimulated the development of IL-10 producing regulatory CD4$^+$LAG3$^+$CD49b$^-$FoxP3$^-$ Tr1 cells. Mechanistically the study indicated that the RA-DCs could suppress allergen-specific Th2 cells via IL-10 and TGF-β production, while IL-27 converted Th2 cells into regulatory Tr1 cells.

Cellular IT is in its infancy within food allergy, and inspiration could be taken from the experiences in the field of autoimmunity, which aims to suppress the pathological Th1/17 response and promote regulatory T cells [65]. Models using other immune cell phenotypes for therapy, as well as optimizing the approach in terms of cell preparation, route of delivery, and adjuvants may be needed.

Conclusion

Animal models of food allergen-specific IT are widely used as a preclinical approach to study the efficacy of new therapeutic strategies. As reviewed here, animal models have been used as a first screen of the applicability of different routes of administration, the use of modified version of allergens, and the use of combining allergen with non-specific immune modulation. Efficacy has mostly been evaluated by means of anaphylactic scores, while the therapeutic mechanisms have primarily been associated of the induction of regulatory T cells, changes in allergen-specific antibody levels, and suppression of Th2-related cytokines. Only few of the reported studies use experimental techniques to ascertain causal therapeutic mechanisms, including the use of signal blocking, cell depletion and transfer experiments, and gene knock-out mice. Thus, there remain opportunities for in-depth mechanistic studies of the induction and maintenance of immunological tolerance in these models. As no allergen-specific immunotherapeutic approaches has yet been accepted in food allergy, animal models may play a valuable tool in addressing safety and efficacy, and to compare different approaches in a controlled environment. Animal models have the unique potential to allow the study of the efficacy of a given approach with different allergens, as well as the in-depth study of mechanisms underlying an immunotherapeutic effect. With the rising appreciation that food allergy is a heterogeneous disease [8] future models should include other disease-relevant clinical outcomes in addition to acute anaphylaxis, including diarrhea, respiratory function and gastrointestinal pathology. Intriguingly, the models presented underline a possible role of allergen-specific differences in therapeutic outcomes, mechanisms, and even sensitization. In line, the skin has been indicated as a route of sensitization for which models has recently been developed [13]. It remains however largely unknown how the route (oral, IP, skin, or respiratory) or method of sensitization (adjuvant use, or genetically susceptible mice) affect therapeutic outcomes in the presented IT models. Animal models will in the future remain a valuable tool to study efficacy and mechanisms in different food allergy phenotypes in a controlled manner.

Conflict of Interest

The authors declares no conflicts of interest in the publication of this review.

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Table 1 - Therapeutic animal models in food allergy

Route	Intervention	Allergen (sensitization)	Host	Sensitization	Treatment	Challenge	Outcomes	Ref.
Oral Immunotherapy (OIT)								
Oral	Egg white	Ovalbumin (Egg)	Mouse; C3H/HeJ	Oral; 1 mg ovalbumin + 10 µg CT weekly, 6 wks	Increasing 1 – 50 mg/day egg white for 14 days	Oral; 50 mg ovalbumin	Short term amelioration of anaphylaxis (temperature loss) and disease score Clinical desensitization lost 2wks post treatment Increased serum ovalbumin-specific IgE and IgA, and similar IgG1 and IgG2a post-treatment Suppression of splenic T cell cytokines (IL-13, IL-10 and IFN-γ) 2 wks post-treatment Treatment induces increased gut permeability	[12]
Oral	Egg white	Ovomucoid (Egg)	Mouse; C3H/HeJ	Oral; 1 mg ovomucoid + 10 µg CT weekly, 6 wks	Increasing 1 – 50 mg/day egg white for 14 days	Oral; 25 mg ovomucoid	Short term amelioration of anaphylaxis (temperature loss) and disease score Desensitization lost after 2 wks post-treatment	[12]
Oral	Native or heated ovomucoid	Ovomucoid (Egg)	Mouse; C3H/HeJ	Oral; 1 mg ovomucoid + 10 µg CT weekly, 6 wks	Increasing 0.5 – 25 mg/day ovomucoid for 14 days	Oral; 25 mg ovomucoid	Abrogation of anaphylaxis and disease score	[12]
Oral	Ovalbumin	Ovalbumin (Egg)	Mouse; C3H/HeJ	Skin; 100 µg ovalbumin weekly, 6 wks	1 mg ovalbumin, daily, 8 wks	Oral; 10, 20, and 50 mg ovalbumin 30 min apart	Short term amelioration of anaphylaxis (temperature loss) Desensitization lost after 5 wks post-treatment Increased serum IgG1 and IgG2a	[13]
Oral	Ovalbumin	Ovalbumin (Egg)	Mouse; C3H/HeJ	Oral; 1 mg ovalbumin + 10 µg CT weekly, 6 wks	1 mg ovalbumin, daily, 8 wks	Oral; 10, 20, and 50 mg ovalbumin 30 min apart	No effect on anaphylaxis (temperature loss) No effect on serum allergen-specific IgG1 and IgG2a	[13]
Oral	Cow milk protein	Cow milk protein	Mouse; BALB/c	Oral; 20 mg cow milk protein + 10 CT weekly, 6 wks	10 µg cow milk protein weekly, 4 or 8 wks	Oral; 10 mg cow milk protein	Clinical scores ameliorated and negative skin test after 8 wks of therapy Decreased serum allergen-specific IgE and IgG1, but similar IgG2a	[14]

							Outcomes	Ref
							Suppression of splenic allergen-specific cytokines IL-5 and IL-13, but unchanged IFN-γ Reduced intestinal expression of IL-5, IL-13 and GATA3, and increased IFN-γ, IL-10, TGF-β and FoxP3 Elevated percentages of intestinal CD4$^+$CD25$^+$FoxP3$^+$ cells expressing IL-10	
Oral	Whey	Whey (milk)	Mouse; C3H/HeOuJ	IG; 20 mg whey + 20 µg CT; day 0, 7, 14, 21 and 28	0.1, 1, 10 or 100 mg whey 5x weekly for 3 wks	50 mg IG, 100 mg IP, or 10 µg ID; whey	Decreased ear swelling, anaphylaxis score and temperature loss following ID challenge Decreased serum mMCP-1 levels following IG challenge No protection from IP-induced anaphylaxis Similar allergen-specific IgE, and increased IgG1 and IgG2a level in serum Reduced CD4$^+$CD69$^+$ST2$^+$ Th2 cells and increased CD4$^+$CD69$^+$CXCR3$^+$ Th1 cells in spleen CD4$^+$CD25$^+$FoxP3$^+$ regulatory T cells increased in mLN, but not spleen Suppressed allergen-specific IL-5 and IL-13, and similar IL-10 and IFN-γ production by splenocytes	[15]
Oral	Peanut butter; Anti-IgE or SYKi facilitated	Peanut butter	Mouse; allergy-prone Il4raF709	Oral; 23 mg peanut butter (5 mg protein) weekly, 4 wks	225 mg peanut butter daily, 3 wks; 25 µg/day anti-IgE IP for 4 days before peanut or 30 mg/kg SYKi IG daily with the peanut	Oral; 430 mg peanut butter	Prolonged suppression of anaphylaxis Expansion of allergen-specific regulatory T cells Suppression of allergen-specific Th2 immune responses	[17]
Oral	Peanut protein extract	Peanut protein	Mouse; C3H/HeOuJ	IG; 6 mg peanut protein extract + 20	0.15, 1.5, or 15 mg peanut	15 mg IG, 100 mg IP,	Decreased ear swelling following ID challenge	[15]

Route	Allergen	Allergen	Model	Sensitization	Treatment	Challenge	Outcomes	Ref
		extract		μg CT; day 0, 1, 2, 7, 14, 21 and 28	protein extract 5x weekly for 3 wks	or 1 μg ID; peanut protein extract	Decreased serum mMCP-1 levels following IG challenge Decreased anaphylaxis score and temperature loss following IP-induced anaphylaxis Similar allergen-specific IgE, and increased IgG1 and IgG2a level in serum Similar CD4$^+$CD69$^+$ST2$^+$ Th2 cells, CD4$^+$CD69$^+$CXCR3$^+$ Th1, and CD4$^+$CD25$^+$FoxP3$^+$ regulatory T cells in spleen Similar allergen-specific IL-5, IL-13, IL-10 and IFN-γ production by splenocytes	
Oral	Peanut protein extract	Peanut protein extract	Mouse; BALB/c	IG; 1 mg peanut protein extract + 10 μg CT weekly for 6 wks	1 mg peanut protein extract week 1, 2 mg protein week 2, and 5 mg protein week 3 - 8.	None	Suppression of allergen-specific IgE and induction of IgG2a Reduced *ex vivo* splenic allergen-specific IL-5 and IL-13, and increased IL-10 Increased splenic CD4$^+$CD25$^+$ regulatory T cells expressing FoxP3, CTLA-4, LAP, CCR9 and CCR4	[16]

Intraperitoneal immunotherapy (IPIT)

Route	Allergen	Allergen	Model	Sensitization	Treatment	Challenge	Outcomes	Ref
IP	Cashew protein extract	Cashew protein extract	Mouse; C3H/HeJ	Oral; 2 mg cashew protein extract + 10 μg CT day 1, 8 and 15, and 5 mg protein + 10 μg CT day 22	3 IP doses weekly; Dosing: 50 μg cashew protein extract week 1, 125 μg week 2, and 200 μg week 3+4	IP; 1 mg cashew protein extract 14 days post treatment	Abrogation of anaphylaxis (temperature loss) and disease score Increased allergen-specific IgG1 and IgG2a, but no effect on IgE Suppression of splenic allergen-specific cytokines IL-5 and IL-13, but unchanged IL-4 and IFN-γ	[21]
IP	Cashew protein extract in cross-reactivity model	Cashew or egg protein extract	Mouse; C3H/HeJ	IP; 500 μg protein extract (cashew or egg) + 2 mg alum week 1, 2 and 4	3 IP doses weekly; Dosing: 100 μg protein extract (cashew) week 1, 250 μg	IP; 1 mg protein extract (cashew, pistachio, or egg)	Abrogation of anaphylaxis (temperature loss) and disease score following cashew or pistachio challenge in cashew-sensitized mice Increased allergen-specific	[22]

							IgG1 Suppression of splenic allergen-specific cytokines IL-4 and IL-5, and increased IL-12p40 Mice sensitized to egg was not protected from egg-induced anaphylaxis following cashew IT	
IP	Cashew or walnut protein extract in multi-sensitization model	Cashew and walnut protein extract	Mouse; C3H/Hej	IP; 500 µg protein extract (cashew and walnut) + 2 mg alum week 1, 2, and 4	3 IP doses weekly; Dosaging: 100 µg protein extract (cashew and/or walnut) week 1, 250 µg week 2, and 500 µg week 3+4	IP; 1 mg protein (cashew, or walnut)	Cashew and cashew + walnut IT protect from cashew-induced anaphylaxis (temperature loss) and disease score Walnut IT partly protect from cashew-induced anaphylaxis (temperature loss) and disease score Walnut and cashew + walnut IT protect from walnut-induced anaphylaxis (temperature loss) and disease score Cashew IT partly protect from walnut-induced anaphylaxis (temperature loss) and disease score Cashew, walnut and cashew + walnut IT increases both cashew and walnut-specific IgG1	[22]

Immunotherapy using hydrolyzed food proteins

IP	Digested cashew protein extract	Cashew protein extract	Mouse; C3H/HeJ	Oral; 2 mg cashew protein extract + 10 µg CT day 1, 8 and 15, and 5 mg protein + 10 µg CT day 22	3 IP doses pr. week; Dosaging: 50 µg digested cashew protein extract week 1, 125 µg week 2, and 200 µg week 3+4	IP; 1 mg cashew protein extract 14 days post treatment	Abrogation of anaphylaxis (temperature loss) and disease score Increased allergen-specific IgG1 and IgG2a, but no effect on IgE Suppression of splenic allergen-specific cytokines IL-5 and IL-13, but unchanged IL-4 and IFN-γ Clinical and immunological outcomes were similar in	[21]

							mice receiving therapy with intact cashew protein	
Oral	Hydrolyzed egg white proteins	Egg white proteins	Mouse; BALB/c	Oral; 5 mg egg white proteins + 10 µg CT 2x weekly, 4 wks	Oral; 5 mg hydrolyzed egg white proteins 3x weekly, 3 wks	Oral; 20 mg egg white proteins	Normalization of serum histamine Decreased allergen-specific IgE and IgG2a, and unchanged IgG and IgG1 Reduced allergen-specific IL-4 and IFN-γ by splenocytes Reduced intestinal expression of IL-4, IL-13, IFN-γ, IL-10 and IL-12p40, and increased IL-18, IL-5, TGF-β and FoxP3	[23]

T cell directed peptide immunotherapy

Oral	Peptides from ovalbumin (immunodominat T cell epitopes) - 3 single peptides or cocktail	Ovalbumin (Egg)	Mouse; BALB/c	Oral; 1 mg ovalbumin + 10 µg CT 2x weekly, 4 wks	Oral; 100 µg peptide 3x weekly, 3 wks	Oral; 20 mg ovalbumin	Peptide cocktail suppressed anaphylactic disease scores and serum histamine levels Reduced allergen-specific IgE and unchanged IgG Allergen-specific decreased IL-4 and increased IFN-γ in splenocytes Reduced intestinal expression of IL-4, IL-5, IL-13, and increased TGF-β and FoxP3 Possibly increased fecal IgA	[34]
Oral	Peptide from ovomucoid (immunodominat T cell epitope) - single or triple sequence	Ovomucoid (Egg)	Mouse; BALB/c	Oral; 1 mg ovomucoid + 10 µg CT 2x weekly, 4 wks	Oral; 1 mg single or 3 mg triple 3x weekly, 4 wks	Oral; 20 mg ovomucoid	Peptide abrogated disease score and histamine release Decreased allergen-specific IgE, similar IgG, IgG1 and increased IgG2a Possibly increased fecal IgA Allergen-specific decreased IL-4 and increased IL-12, IFN-γ and IL-10 in splenocytes Increase percentage of blood CD4$^+$FoxP3$^+$ cells	[35]
Oral	Peptides from shrimp-tropomyosin (immunodominat T cell epitopes) - Cocktail of 6	Recombinant shrimp-tropomyosin	Mouse; BALB/c	Oral; 100 µg recombinant shrimp-tropomyosin + 10 µg CT day 0, 12, 19, and 26	Oral; 1,2 mg peptide mix 2x weekly, 4 wks	Oral; 500 µg recombinant shrimp-tropomyosin	Peptide cocktail suppressed disease scores, diarrhea, and serum mMCP-1 levels Decreased allergen-specific IgE, increased IgG2a and	[28]

							similar IgG1 in serum Suppression of intestinal IL-5 and IL-13 expression, and increased FoxP3, CD25, IL-10, IL-12, IL-18 and IFN-γ Allergen-specific decreased IL-4 and IL-5, and increased IFN-γ in splenocytes	
peptides								
Oral	Peptides from beta-lactoglobulin (immunodominat T cell epitopes) - 2 single peptides	beta-lactoglobulin (Milk)	Mouse; BALB/c	IP; 50 µg beta-lactoglobulin + 2 mg alum weekly, 3 wks	Oral; 1 mg peptide 3x weekly, 4 wks	Oral; 50 mg beta-lactoglobulin	Peptides abrogation of anaphylaxis (temperature loss) and suppressed disease scores No effect on serum allergen-specific IgE, IgG1, IgG2a, IgA, total-IgE, or fecal total-IgA No differences in allergen-specific IFN-γ, IL-12, IL-4 and/IL-10 in splenocytes or mLN cells No difference in splenic CD4$^+$CD25$^+$FoxP3$^+$ regulatory T cell population	[36]

Subcutaneous immunotherapy (SCIT)

SC	Whey	Whey (milk)	Mouse; C3H/HeOuJ	IG; 20 mg whey + 20 µg CT; day 0, 7, 14, 21, and 28	SC; 2.5, 10 or 25 µg whey 3x weekly, 3 wks	50 mg IG, 100 mg IP, or 10 µg ID; whey	Decreased anaphylaxis score and temperature loss, but minimal effect on ear swelling following ID challenge Decreased serum mMCP-1 levels following IG challenge No protection from IP-induced anaphylaxis Increased allergen-specific IgE, IgG1, and IgG2a levels in serum Reduced CD4$^+$CD69$^+$ST2$^+$ Th2 cells and increased CD4$^+$CD69$^+$CXCR3$^+$ Th1 cells in spleen Similar CD4$^+$CD25$^+$FoxP3$^+$ regulatory T cells in mLN and spleen Similar allergen-specific IL-5, IL-13, IL-10, and IFN-γ	[15]

							production by splenocytes	
SC	Peanut protein extract	Peanut protein extract	Mouse; C3H/HeOuJ	IG.; 6 mg peanut protein extract + 20 µg CT; day 0, 1, 2, 7, 14, 21, and 28	SC; 1, 10 or 100 µg peanut protein extract 3x weekly, 3 wks	15 mg IG, 100 mg IP, or 1 µg ID; peanut protein extract	Minimal effect on ear swelling following ID challenge Decreased serum mMCP-1 levels following IG challenge Decreased anaphylaxis score and temperature loss following IP-induced anaphylaxis Increased allergen-specific IgE, IgG1, and IgG2a levels in serum Increased $CD4^+CD69^+ST2^+$ Th2 cells, and similar $CD4^+CD69^+CXCR3^+$ Th1, and $CD4^+CD25^+FoxP3^+$ regulatory T cells in spleen Increased allergen-specific IL-5 and IL-10, and similar IL-13 and IFN-γ production by splenocytes	[15]

Epicutaneous immunotherapy (EPIT)

EP	Ovalbumin	Ovalbumin (Egg)	Mouse; C3H/HeJ	Skin; 100 µg ovalbumin weekly, 6 wks	100 µg ovalbumin in Viaskin patch 48 hours weekly, 8 wks	Oral; 10, 20, and 50 mg ovalbumin 30 min apart	Long-term reversal of anaphylaxis (temperature loss) at 1 and 5 weeks post-treatment Persistent increased serum allergen-specific IgG1 and IgG2a at 1 and 5 weeks post-treatment No effect in basophil activation tests No therapeutic blocking antibodies demonstrated by passive sensitization of naïve animal Therapeutic induction of $CD4^+LAP^+FoxP3^-$ regulatory T cells in skin dLN and mLN expressing CCR9, CCR6 and CCR4 Therapeutic effect mediate by TGF-β	[13]

EP	Ovalbumin	Ovalbumin (Egg)	Mouse; C3H/HeJ	Oral; 1 mg ovalbumin + 10 µg CT weekly, 6 wks	100 µg ovalbumin in Viaskin patch 48 hours weekly, 8 wks	Oral; 10, 20, and 50 mg ovalbumin 30 min apart	Reversal of anaphylaxis (temperature loss) Increased allergen-specific IgG1 and IgG2a at 1 week, but not 5 weeks post treatment No effect in basophil activation tests No therapeutic blocking antibodies demonstrated by passive sensitization of naïve animal Therapeutic induction of CD4$^+$LAP$^+$FoxP3$^-$ regulatory T cells in skin bLN and mLN expressing CCR9, CCR6 and CCR4 Therapeutic effect mediate by TGF-β	[13]
EP	Peanut protein extract	Peanut protein extract	Mouse; BALB/c	Oral; 1 mg peanut protein extract + 10 µg CT weekly, 6 wks	100 µg peanut protein extract in Viaskin patch 48 hours weekly, 8 wks	Peanut as feed day 1-4, peanut in feed day 5-10, and 10 mg IG daily the last 3 days	Suppression of esophageal eosinophilia, and acanthosis and inflammation scores Reversal of allergic enteropathy and villous morphology Increased IgG2a in serum Reduced allergen-specific IL-5, IL-13 and IL-10, and similar IFN-γ production by splenocytes FoxP3 mRNA expression in esophagus Increased splenic CD4$^+$CD25$^+$FoxP3$^+$ regulatory T cells expressing CTLA-4, but not IL-10 and PD-1 Reduced eotaxin and increased FoxP3 expression in esophagus Efficacy dependent on CD25 during treatment Long-term stability of treatment induced regulatory T cells was demonstrated by adoptive transfer of CD4$^+$CD25$^+$ T cells 8 wks post	[45,46]

							treatment	
EP	Peanut protein extract	Peanut protein extract	Pig; (LW x LR) x P	IP; 100 µg peanut protein extract + 8 mg alum week 1, 2, and 4	100 µg peanut protein extract in Viaskin patch for 24 hours daily, 81 days	Oral; 4 mg peanut protein extract daily for 10 days	Reversal of visual gastritis, and gastric tissue inflammation and eosinophilia Reduced serum allergen-specific IgE Suppression of splenic Gata3, FoxP3, IL-5 and eotaxin mRNA expression	[47]
Sublingual immunotherapy (SLIT)								
SL	Cow milk protein	Cow milk protein	Mouse; BALB/c	IG; 20 mg cow milk protein + 10 µg CT weekly, 6 wks	10 pg 2x weekly, 4 weeks. Followed by 10 ng 2x weekly, 4 weeks; cow milk protein	IG; 10 mg cow milk protein twice 24h apart	Suppression of clinical symptom scores Decreased allergen-specific serum IgE Decreased allergen-specific IL-5 production by splenocytes Increased IL-10 and TGF-β levels in jejunum	[52]
Immunotherapy using immunomodulatory adjuvants								
Oral	TLR9 agonist (IMO) + peanut protein extract	Peanut protein extract	Mouse; C3H/HeJ	Oral; 1 mg peanut protein extract + 10 µg CT day 0 and 14.	1 mg peanut protein extract with or without 150 µg TLR9 agonist day 21, 24, 28, and 31	IG; 1 mg peanut protein extract 2x, 30-40 min interval	Suppression of anaphylaxis scores and reversal of serum histamine levels Reduced mast cells and eosinophils in small intestine Decreased in allergen-specific IgE and IgG1, and increased IgG2a in serum Increased allergen-specific IgA in intestine Suppressed allergen-specific IL-5 and IL-13, and increased IFN-γ production by splenocytes	[54]
IG	TLR9 agonist (class B CpG-ODN 1826) + defatted whole roasted peanut in PLGA nanoparticles	Whole roasted peanut	Mouse; C3H/HeJ	IG; 10 mg whole roasted peanut + 20 µg CT + 1.1 µl 80-proof EtOH pr. g BW weekly, 6 wks. 50 mg boost with same adjuvant wks 7 and 8	200 µg whole roasted peanut with or without 1.8 µg TLR9 agonist weekly, 4 wks	Oral; 200 mg whole roasted peanut (done weekly with IG sensitization	Suppression of anaphylaxis scores, temperature loss and plasma histamine levels Decrease in allergen-specific IgE and IgG1, and increased IgG2a in serum Decreased allergen-specific IL-4, IL-5 and IL-13, and	[55]

						dose the day after for 5 wks)	increased IFN-γ production by splenocytes	
IP	TLR9 agonist (class A CpG-ODN 1585, class B CpG-ODN 1826, or class C CpG-ODN M362) + peanut protein extract	Peanut protein extract	Mouse; C3H/HeJ	IP; 500 µg peanut protein extract + 2 mg alum weekly, 3 wks	Increasing 100 – 500 µg peanut protein extract with or without 30 µg TLR9 agonist twice weekly, 3 wks	IP; 1 mg peanut protein extract	Class B and C CpG-ODNs decreased anaphylaxis scores, temperature loss and serum MMCP-1 levels Similar allergen-specific IgE and IgG1, and increased IgG2a in serum Decreased allergen-specific IL-5 and IL-13, and similar IL-4 production by splenocytes Class B CpG-ODN increased allergen-specific IFN-γ production by splenocytes	[56]
ID	TLR9 agonist (class B CpG-ODN 1826) + ovalbumin in PLGA nanoparticles	Ovalbumin (Egg)	Mouse; BALB/c	IP; 50 µg ovalbumin + 2 mg alum weekly, 2 wks	20 µg ovalbumin with or without 25 µg TLR9 agonist, 3x 6 days apart	IP; 1 mg ovalbumin	Limited effect on anaphylaxis score and temperature loss Possible protection from death	[57]
SC	Heat-killed Listeria monocytogenes (HKLM) + IgE-binding modified recombinant Ara h 1-3	Whole roasted peanut	Mouse; C3H/HeJ	IG; 10 mg whole roasted peanut + 20 µg CT + 1, weekly, week 1-6 and 8	90 ug modified recombinant Ara h 1-3 with 10^8 HKLM weekly, 3 wks, or without HKLM 3x weekly, 4 wks	IG; 25 mg whole roasted peanut 2x, 30 - 40 min interval	Suppression plasma histamine levels, but limited effect on of anaphylaxis scores and temperature loss Limited effect on respiratory distress measured as unrestrained Peak Expiratory Flow Decreased allergen-specific IgE and increased IgG2a in serum Decreased allergen-specific IL-4, IL-5 and IL-13, and increased IFN-γ production by splenocytes	[58]
PR	Heat-killed Escherichia coli expressing IgE-binding modified recombinant Ara h 1-3	Whole roasted peanut	Mouse; C3H/HeJ	IG; 10 mg whole roasted peanut + 20 µg CT + 1, weekly, week 1-6 and 8	0,9, 9 or 90 µg whole roasted peanut weekly, 3 wks	IG; 25 mg whole roasted peanut 2x, 30-40 min interval at 2, 6 and 10	Long-term reversal of anaphylaxis scores and suppression of plasma histamine levels Decreased allergen-specific IgE and increased IgG2a in serum	[59]

							wks post-treatment	Decreased allergen-specific IL-4, IL-5 and IL-13, and increased IFN-γ and TGF-β production by splenocytes	
IN	Nanoemulsion (cetylpyridinium chloride and oil based) + peanut protein extract	Peanut protein extract	Mouse; C3H/HeJ	IP; 20 µg peanut protein extract + 2 mg alum, weekly, 2 wks	20 µg peanut protein extract with or without 20 % nanoemulsion, 3x, 4 wks apart	IG; 10 mg peanut flour, 6x during 2 wks (or 100 µg IP)	Suppression of anaphylaxis scores and temperature loss following IP challenge Suppression of anaphylaxis scores and serum MCPT-1 levels following IG challenge Decreased allergen-specific IgE and IgG1, and increased IgG2a and IgG2b Decreased allergen-specific IL-4, IL-13, IL-33 and IL-21, and increased IFN-γ, IL-22, and IL-10 in mLN cells Increased percentage of CD4+CD25+FoxP3 regulatory T cells in mLN Induction of therapeutic efficacy was partly dependent on IL-10	[61]
	Nanoemulsion (cetylpyridinium chloride and oil based) + peanut protein extract	Peanut protein extract	Mouse; C3H/HeJ	IG; 1 mg peanut protein extract + 10 µg CT, 3x weekly, 2 wks	20 µg peanut protein extract with or without 20 % nanoemulsion, 3x, 4 wks apart	IG; 10 mg peanut flour, 6x during 2 wks (or 100 µg IP)	Suppression of anaphylaxis scores and serum MCPT-1 levels following IG challenge Decreased allergen-specific IL-4, and IL-13, and increased IFN-γ and IL-10 in mLN cells	[61]
SL	IL-2/anti-IL-2Ab complex (IL-2C) + cow milk protein	Cow milk protein	Mouse; BALB/c	IG; 20 mg cow milk protein + 10 µg CT weekly, 6 wks	10 pg cow milk protein 2x weekly, 4 weeks. Followed by 10 ng cow milk protein 2x weekly, 4 weeks. With or without IL-2C (15000IU IL-2 + 4.5 µg anti-IL-2) IP 5x in the	IG; 10 mg cow milk protein twice 24h apart	Possibly enhanced SLIT therapy indicated by weak suppression of clinical symptom scores and allergen-specific serum IgE Expansion of intestinal mucosal CD4+CD25+FoxP3+ regulatory T cells Increased TGF-β levels in jejunum	[52]

					treatment period		
IV	Peanut protein extract fixed to splenic leukocytes	Peanut protein extract	Mouse; C3H/HeJ	IG; 100 µg peanut protein extract + 50 µg SEB, weekly, 8 wks	5×10^7 leukocytes, weekly, 2 wks	Oral; 5 mg peanut protein extract	Weak effect on anaphylaxis scores, but reversed blood eosinophilia Similar allergen-specific IgE Decreased allergen-specific IL-4 and IL-13, and similar IL-5 production by splenocytes	[62]

Cellular immunotherapy

IV	CD4+CD25+ regulatory T cells induced by OIT, SLIT or EPIT	Peanut protein extract	Mouse; BALB/c	IG; 1 mg peanut protein extract + 10 µg CT weekly for 6 wks	5×10^5 Treg cells	Peanut as feed day 1-4, peanut in feed day 5-10, and 10 mg peanut protein extract IG daily the last 3 days	EPIT-induced CD4$^+$CD25$^+$ Tregs suppressed of esophageal eosinophilia Suppressed allergen-specific IL-5, IL-13, IFN-γ and IL-10 production by splenocytes	[16]
IP	Ovalbumin-pulsed retinoic acid-differentiated dendritic cells (RA-DCs) generated *in vitro*	Ovalbumin (Egg)	Mouse; BALB/c	IP; 2 µg ovalbumin + 2 mg alum	10^6 RA-DCs	IG; 2 mg ovalbumin	Suppression of anaphylaxis score, diarrhea incidence, and serum mMCP-1 Decreased serum allergen-specific IgE and IgG1 Decreased IL-4, IL-5, IL-9 and IL-13 in peritoneal fluid Therapeutic effect dependent on IL-27 production by RA-DCs	[64]
IP	Peanut protein-pulsed retinoic acid-differentiated dendritic cells (RA-DCs) generated *in vitro*	Peanut protein extract	Mouse; BALB/c	IP; 20 µg peanut protein extract + 1 mg alum	10^6 RA-DCs	IG; 50 mg peanut butter	Suppression of anaphylaxis score, diarrhea incidence, and serum mMCP-1 Decreased serum allergen-specific IgE and IgG1 Similar IL-4, IL-5, IL-9 and IL-13, and increased IL-10 in peritoneal fluid	[64]

CMP, Cow's milk protein; CT, Cholera toxin; dNL, Draining lymph node; EP, Epicutaneous; EPIT, Epicutaneous Immunotherapy; HK, Heat-killed; ID, Intradermal; IN, Intranasal; IG, Intragastric; IP, Intraperitoneal; IPIT, Intraperitoneal Immunotherapy; IT, Immunotherapy; IV, Intravenous; mLN, Mesenteric lymph node; PR, Per rectum; SC, Subcutaneous; SCIT, Subcutaneous immunotherapy; SL, Sublingual; SLIT, Sublingual IT; Treg, Regulatory T cell.

Figure legends

Figure 1 – Overview of allergen-specific immunotherapy models in food allergy. Flowcharts show models in relation to a particular food allergy (egg, cow's milk, peanut, tree nut, and shrimp) with the specific allergen, mode of sensitization, and therapy used. The thickness of lines shows the number of models reported using the specific approach (1-8). Immunotherapeutic approaches include oral (OIT), intraperitoneal (IPIT), subcutaneous (SCIT), epicutaneous (EPIT), or sublingual (SLIT) administration of allergen, allergen in combination with immunomodulatory adjuvant (adjuvant IT) or immune cells (cellular IT), and preparations of hydrolyzed allergen or allergen-derived T cell epitopes. Unless stated otherwise the reported models were conducted in mice. Il4raF709 designate that the model was conducted in allergy-prone mice with gain-of-function mutation in the IL-4 receptor α chain.

Figure 2 – Overview of clinical and immunological outcomes assessed within allergen-specific immunotherapy models in food allergy. Outcomes has been divided into clinical (symptoms), para-clinical (histology), and immunological (serology, systemic and tissue immune responses) measures.

OUTCOME PARAMETERS

SYMPTOMS

Anaphylaxis
- Severity scoring
- Temperature loss
- Death incidence
- Gut permeability
- Mast cell degranulation by serum mMCP-1 or histamine

Diarrhea
- Severity scoring
- Incidence

Respiratory distress
- Peak Expiratory Flow

Gastritis
- Visual scoring

HISTOLOGY

Esophageal pathology
- Inflammation scoring
- Eosinophilia
- Acanthosis

Intestinal pathology
- Inflammation scoring
- Eosinophilia
- Mastocytosis
- Villous morphology

Gastric pathology
- Inflammation scoring
- Eosinophilia

SEROLOGY

Antibody levels
- Serum allergen-specific IgE, IgG1, IgG2a, and IgA
- Serum total-IgE
- Intestinal/fecal allergen-specific IgA and total-IgA

Antibody function
- Ear swelling test
- Basophil activation test
- Passive sensitization by serum transfer

SYSTEMIC IMMUNE RESPONSE

Splenic T cell response
- Allergen-specific type-2 (IL-4, IL-5, IL-13), type-1 (IFNγ, IL-12p40) and regulatory (IL-10 and TGFβ) cytokine production *ex vivo*
- Spleen type-2 (IL-5, IL-13, GATA3, eotaxin), type-1 (Tbet) and regulatory (FoxP3) mRNA gene expression

Splenic T cells
- CD4$^+$CD69$^+$ST2$^+$ Th2 cells
- CD4$^+$CD69$^+$CXCR3$^+$ Th1 cells
- CD4$^+$CD69$^+$FoxP3$^+$ Treg cells
- CD4$^+$CD25$^+$ Treg cells
- CD4$^+$CD25$^+$FoxP3$^+$ Treg cells
- IL-10, CTLA-3, PD-1, LAP, CCR9 and CCR4 expression by Treg cells

Blood T cells
- CD4$^+$FoxP3$^+$ Treg cells

TISSUE IMMUNE RESPONSE

mLN T cell response
- Allergen-specific type-2 (IL-4), Type-1 (IFNγ, IL-12p40) and regulatory (IL-10) cytokine production *ex vivo*

mLN T cells
- CD4$^+$CD69$^+$ST2$^+$ Th2 cells
- CD4$^+$CD69$^+$CXCR3$^+$ Th1 cells
- CD4$^+$CD69$^+$FoxP3$^+$ Treg cells
- CD4$^+$LAP$^+$FoxP3$^-$ Treg cells
- CCR9, CCR6 and CCR4 expression by Treg cells

Intestinal T cell response
- Intestine type-2 (IL-4, IL-5, IL-13, GATA3), type-1 (IFNg, IL-18) and regulatory (IL-10, TGFβ, CD25, FoxP3) mRNA gene expression
- Type (IL-4, IL-5, IL-9, IL-13) and regulatory (IL-10) cytokine levels in IP fluid

Intestinal T cells
- CD4$^+$CD25$^+$FoxP3$^+$ Treg cells
- IL-10 expression by Treg cells

Esophageal T cell response
- Type-2 (IL-5, eotaxin), type-1 and regulatory (FoxP3) mRNA gene expression