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REVIEW



Current Trend in Immunotherapy for Peanut Allergy

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ABSTRACT

Peanut allergy is a hypersensitivity reaction with symptoms varying from mild to severe anaphylaxis, tends to be lifelong and very few are able to outgrow this allergy. The prevalence of peanut allergy is highest among the Western countries and over the past decade, a 3.5 fold increase in prevalence of peanut allergy was reported among children in the United States. Increasing prevalence has also been observed among the Asian countries. As with other food allergies, peanut allergy reduces quality of life for the affected individuals and the social and economy burden of healthcare for peanut allergy is substantial. To date, there is no effective treatment for peanut allergy and disease management is by avoidance or relieve of symptoms via administration of epinephrine. Peanut allergy is a type-1 hypersensitivity reaction due to specific IgE production by activated T-helper type 2 (T_H2) cells. Studies on various immunotherapy routes such as oral immunotherapy (OIT), sublingual immunotherapy and epicutaneous immunotherapy trials using peanut have shown the ability to induce desensitisation, shifting the allergen-specific cytokine production away from a T_H2 respond. In the recent years, lactic acid bacteria probiotics have been reported to down-regulate allergy due to its inherent immunomodulatory properties. Wild-type probiotic in combination with peanut proteins or recombinant probiotics harbouring peanut allergens have been explored for OIT due to its ability to down-regulate allergen-specific-IgE production and the T_H2 responses, while increasing the beneficiary population of T_H1 regulatory T cells (T_{reg}). This review discusses the current strategies in immunotherapy for peanut allergy.

Abbreviations: CRD: component resolved diagnosis; DC: dendritic cells; Foxp3: Forkhead box P3; FPE: food protein-induced enteropathy; FPIAP: food protein-induced allergic proctocolitis; FPIES: food protein-induced enterocolitis syndrome; GRAS: generally recognised as safe; hKLM: heat-killed *Listeria monocytogenes*; LAB: lactic acid bacteria; OIT: oral immunotherapy; SCIT: subcutaneous immunotherapy; SLIT: sublingual immunotherapy; SPT: skin-prick testing; TFH: follicular T-helper cells; TGFβ: transforming growth factor β; T_H1: T-helper type 1; T_H2: T-helper type 2; TLR: toll-like receptor; TSLP: thymic stromal lymphopoietin

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Background

Food allergy is an important public health problem that affects children and adults worldwide. Unlike food intolerance that is a common reaction and is a less serious condition, food allergy is an abnormal response to a food triggered by the body's immune system.¹ Even a small amount of the allergy-causing food can trigger symptoms ranging from merely digestive problems, hives or swollen airways to a life-threatening anaphylaxis reaction. Sensitisation to food allergens can occur either in the gastrointestinal tract (class I food allergy) or as a consequence of cross reactivity to structurally homologous inhalant allergens (class II food allergy). Food allergy can be

broadly divided into those that are IgE mediated and those that are non-IgE mediated, the former results in acute onset of symptoms after food ingestion. The IgE-mediated food allergy can be either class I, involves primary sensitisers that occurs in the gastrointestinal tract and accounted for systemic reactions and or class II food allergy caused by cross reactivity to structurally homologous inhalant allergens.¹ Milk (casein), egg (ovomucoid), and peanut allergens (Vicillin, conglutin, glycinin) are class I, food allergens which are highly stable and can retain under acidic, heat and protease condition, whereas class II allergens (example Bet v1) are heat labile and susceptible to digestive process.

Peanut allergy is an IgE-mediated type I hypersensitivity reaction caused by production of allergen-specific IgE antibodies as a result of exposure to peanut allergen and can be a potentially life-threatening food allergy.^{2,3} The prevalence of peanut allergy has significantly increased for the past two decades and is currently affecting more than 1% of children in the Western countries. In addition, a 3.5 fold increase in peanut allergy among children in the United States within a period of 11 years has been reported.⁴⁻⁶ Although peanut allergy is more prevalent in Western countries, there has been an increasing prevalence among children and adolescents in Asian countries such as Taiwan (1.10% of children aged below 3 years old), Hong Kong (0.52% of children aged 2–7 years old), Japan (0.26% of children below 6 years old) and Philippines (0.43% of adolescents aged 14–16 years old).⁷ A study on food protein sensitisation patterns in Singapore reported 27.3% of 227 children diagnosed with food allergy were allergic to peanut.⁸ Children with multiple food hypersensitivities and a family history of atopic dermatitis were found more likely to develop peanut allergy. A study on clinical manifestation and sensitisation of allergic children from Malaysia reported that among 192 allergic children (35 children aged <2 years; 157 children aged 2–10), reactivity towards peanut-specific IgE was 36.4% for children aged <2 years and 12.6% for children aged 2–10.⁹

Peanut allergy is often a lifelong condition and very few children are able to outgrow this allergy.^{10,11} To date, there is still no absolute cure for peanut allergy, albeit effort on peanut immunotherapy has shown promising result. Avoidance management and administration of epinephrine for relief of symptoms is still the recommended practice.⁶ The standard diagnostic approach commonly used to determine sensitivity towards peanut includes combination of patient's clinical history with skin-prick testing (SPT) and specific IgE immunoassay.¹² Component resolved diagnosis (CRD) uses recombinant allergens of higher purity and consistency which offers a better recognition of specific IgE compared to the use of crude peanut extracts.¹³

Mucosal immunity

The largest reservoir of immune cells in the body resides in the gastrointestinal tract and serves as a barrier from invading pathogens and foreign antigens. Single columnar layer epithelial cells in the gut secreting antimicrobial peptides, mucins and trefoil factors confer innate immune response. Immune cells residing below the epithelial in inductive sites comprised of CD4⁺ and CD8⁺ T effector and regulatory cells,

dendritic cells, macrophages and antibody secreting B which provide specific adaptive immune response to invading pathogens and foreign antigens.¹⁴ The inductive sites include organised secondary lymphoid organs such as the mesenteric lymph nodes, cryptopatches and Peyer's patches while a more diffuse lymphoid compartment comprising of lamina propria and epithelial. Soluble antigens are taken up through the villi around the epithelium lining and carried to the mesenteric lymph nodes, while particulate antigens are taken up by specialised epithelial cells called M cells located near the Peyer's patches.¹⁴ One of the challenges in mucosal immunity is the ability to differentiate the harmful pathogens and harmless or beneficial commensal organism. As such the antigen-presenting cells (dendritic cells and macrophages) in the gut are hypo-responsive, able to secrete high levels of immunoregulatory cytokines such as IL-10 and the mechanism for immune reactivity towards microbes or food antigens may be quite different in the gut-associated lymphoid tissue compared to other lymphoid tissues.¹⁵

Mucosal immunity and peanut allergy

Sensitisation

Allergen presentation and allergen-specific-IgE antibody production are the two major components in the immune mechanisms of peanut allergy. Sensitisation to peanut allergen may occur either via cutaneous exposure or oral route through peanut allergen ingestion as shown in Figure 1. The allergen will be taken up by the antigen-presenting cells and presented via MHC class II to the CD4⁺ cells to develop T_H2 cells. In the cutaneous exposure to peanut allergen, skin epithelium will produce pro-inflammatory cytokines including IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) that drives resident dendritic cells (DCs) and immune cells towards a T_H2-dependent sensitisation to peanut allergen.^{16,17} The activated DCs may secrete retinoic acid that induces allergen-specific T_H2 cells to express gut-homing markers; these are the cells responsible for inflammatory T_H2 cell-mediated response when orally challenged with peanut allergens.^{18,19} During sensitisation via oral route, the allergen may enter through the microfold cells (M cells) or through damaged mucosal caused by inflammation in the gut. The activated gut epithelium and Tuft cells will secrete proinflammatory cytokines such as IL-5, IL-13, IL-25, IL-33 and TSLP thereby initiating a T_H2 cell-mediated immune response leading to tissue eosinophils and basophils

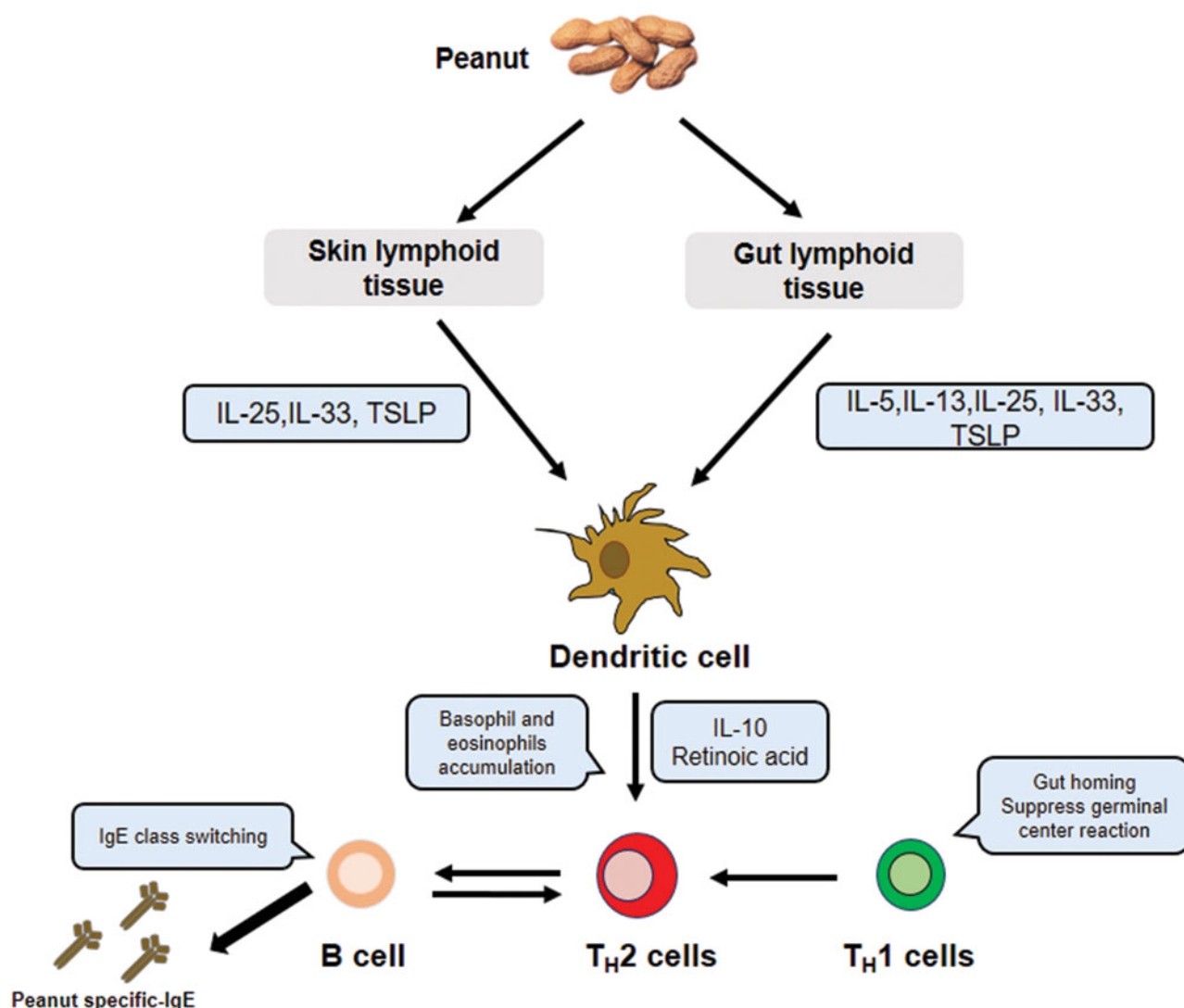


Figure 1. Mechanism of peanut sensitisation. Initial sensitisation of peanut allergen is thought to be through the GI track and skin epithelium. Upon allergen exposure, the proinflammatory cytokines drive the dendritic cells and immune cells towards TH2 cells. Peptides are then presented to TH2 cells, leading to activation of B cells that eventually mature into IgE-producing plasma cells.

accumulation and IgE class-switching by B cells.^{20,21} (Figures 1 and 2). Upon re-encounter, either via oral or cutaneous exposure, the allergen epitopes will attach to allergen-specific-IgE molecules bound to FcεRI receptors on the surface of effector cells (e.g. mast cells and basophils) and this cross-linking of IgE-bound receptors will lead to the release of pre-formed histamine and other inflammatory mediators of the immediate allergic reaction.²¹ Following this immediate phase of the response, the production of leukotrienes, platelet activating factor and cytokines such as IL-4, IL-5 and IL-13 help to maintain allergic inflammation resulting in rapid manifestation of the allergic symptoms. The inflammatory cytokines induce vascular dilation, smooth muscle contraction and tissue damage. Symptoms manifestation may differ depending on the site of exposure, either in

gastrointestinal (oral tingling, pruritus and/or swelling, as well as nausea, abdominal pain and/or vomiting), in respiratory (wheezing and/or airway inflammation) or on the skin (flushing, urticaria, angioedema and/or pruritus). Systemic responses such as hypotension caused by fluid leakage from the vasculature and/or hypothermia and anaphylaxis may also occur, the latter being the most serious and rapid allergic reaction involving multiple organ systems.²² In non-allergic individuals, mast cells are coated with polyclonal IgE with random specificities thereby no cross-linking can take place to a specific allergen.

Food tolerance and desensitisation

The most essential features of the mucosal immunity are suppression. Suppression can be achieved by

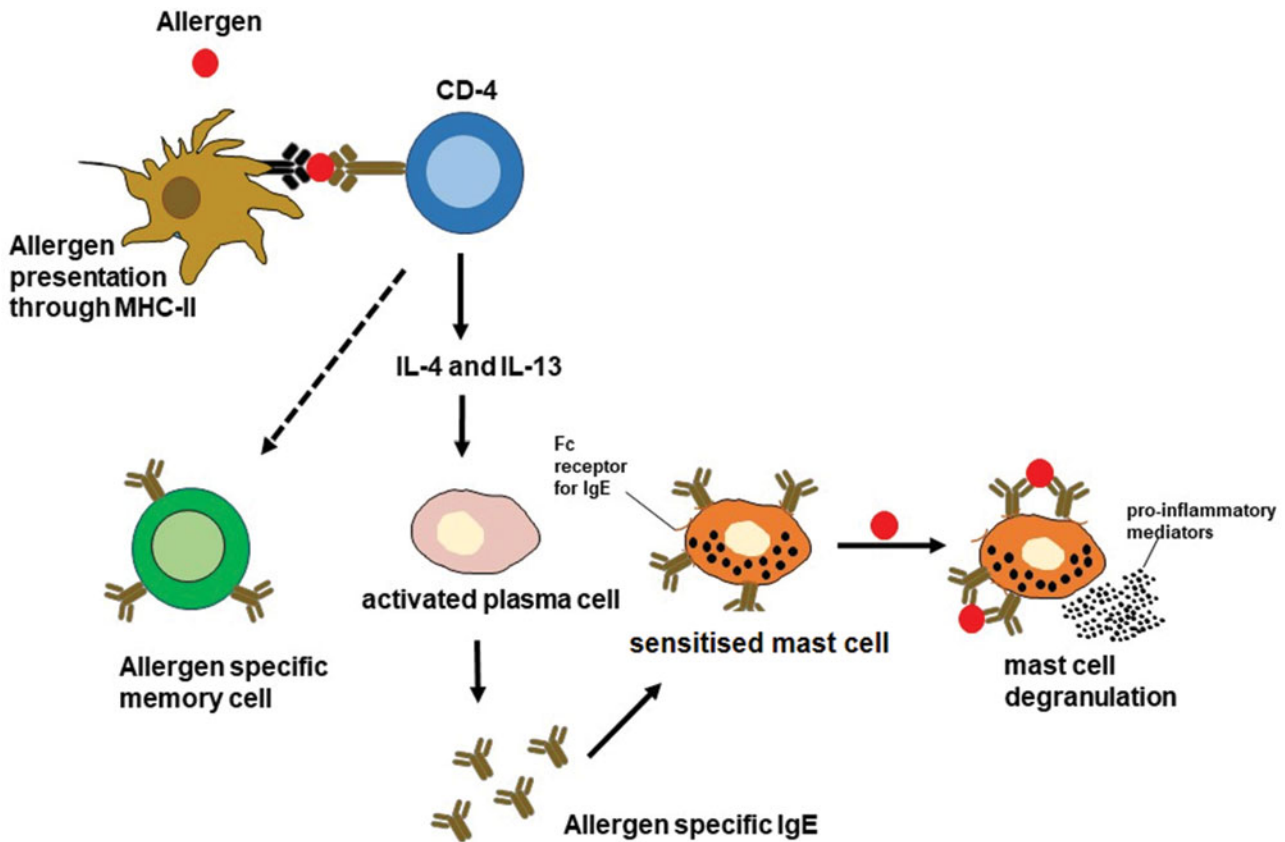


Figure 2. General mechanism underlying an immediate type 1 hypersensitivity reaction. The immunologic mechanism of type 1 hypersensitivity reaction starts when allergen penetrates the mucosal barrier for the first time. The allergen is taken up by the antigen-presenting cells (APC) that expresses MHC class II for presentation to CD4⁺ cells. Activated CD4⁺ cells in the presence of IL-4 and IL-13 will be differentiated to allergen-specific TH2 cells, some of which will develop into memory cells. The activated TH2 cells produce IL-4 and IL-13 cytokines that induces allergen-specific B cells to proliferate and differentiate into plasma cells producing allergen-specific IgE antibodies that can bind to the surface of basophils and mast cells. Upon re-encounter with the same allergen, the allergens will cross-link with allergen-specific IgE bound to high affinity IgE receptor (FcεRI) on the mast cells and eventually lead to basophils and mast cells degranulation. A whole array of chemical mediators will be released such as the histamines, leukotriene, numerous cytokines and chemokines conferring the allergic effect.

two phenomena: the controlled low grade inflammation and the oral tolerance induction.¹⁵ The development of tolerance in a healthy immune system is achieved by the unresponsiveness towards potential allergens even in the absence of regular allergen exposure. The process begins with the uptake of allergens in the gut by M cells-mediated transcytosis or direct allergens sampling by CX₃CR1⁺ macrophages which transfers the captured allergen to CD103⁺ DCs as shown in Figure 3(A).²³ The activated CD103⁺ DC secretes transforming growth factor-β (TGF-β) and retinoic acid as it migrates from the lamina propria to the draining mesenteric lymph nodes where it presents the allergen to naïve T cells to induce differentiation into forkhead box P3 (Foxp3) regulatory T (T_{reg}) cells.^{24–26} Moreover, these CD103⁺ DCs are able to secrete IL-10 which helps in maintenance of the Foxp3 T_{reg} cells, IL-10-secreting T_{reg} cells and TGF-β-secreting T_H3 cells.

The retinoic acid produced by CD103⁺ DC also induces the secretion of integrin α4β7 for gut homing of these T_{reg} cells to exert its regulatory role in the immune response.²⁷ The hypomethylation of the Foxp3 locus of T_{reg} cells plays an essential role in inducing and maintaining the functional tolerance induced by oral immunotherapy (OIT).²⁸ Moreover, the inhibition of IL-4 receptor further enhances tolerance and augments the T_{reg} in the mouse model. Oral exposure to high dose allergens has been shown to cause anergy or deletion of antigen-specific T cells, possibly after DC interaction.²⁹ Follicular T-helper cells (TFH) secrete cytokines which induce B cells class-switch to produce a particular antibody isotypes (IgA in tolerance and IgG4 in desensitisation), whereas follicular T_{reg} cells suppress the germinal centre reaction.³⁰

On the contrary, desensitisation is termed as the reversible state of tolerance whereby short-term

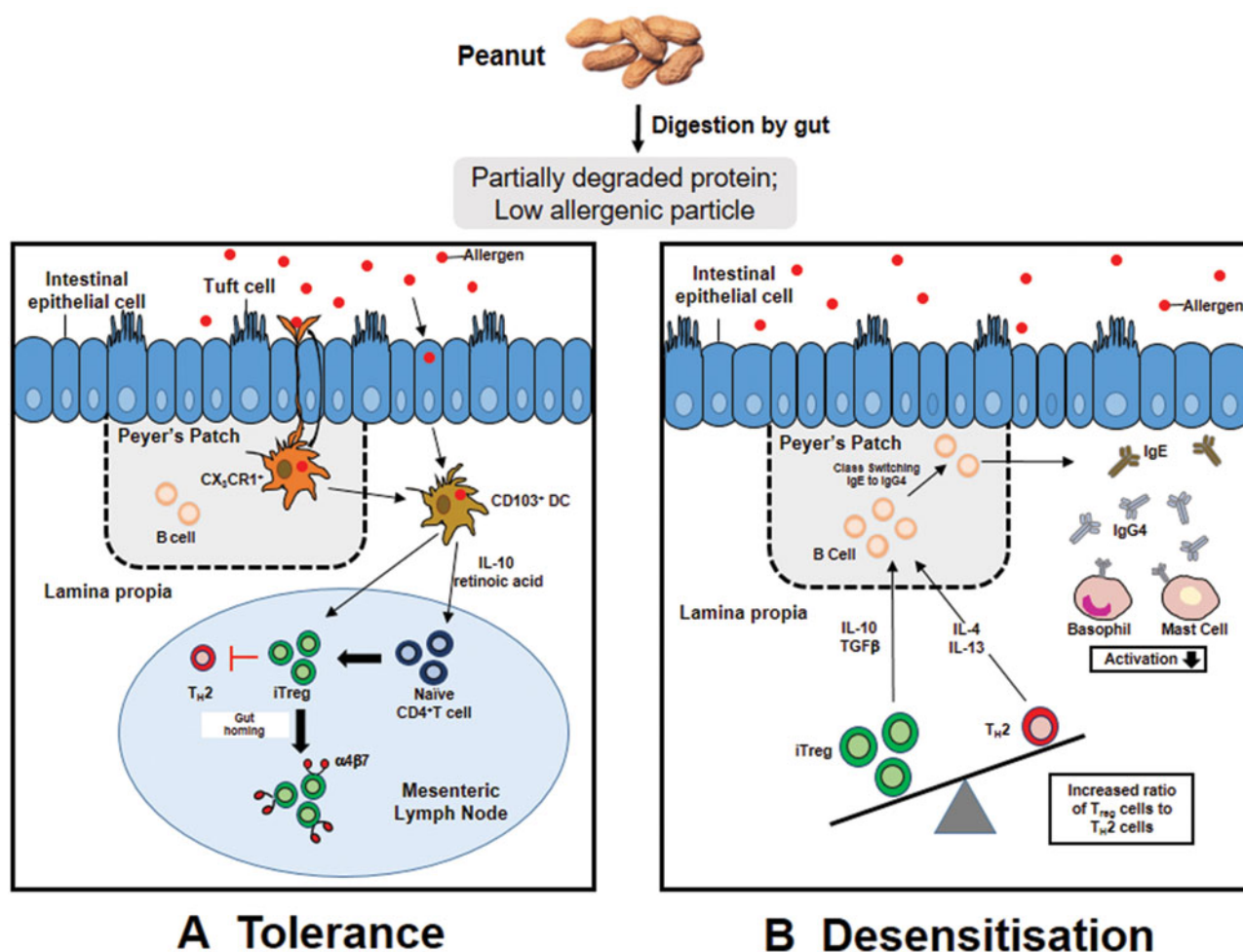


Figure 3. Molecular mechanism of immune tolerance and desensitisation of peanut allergen in gut. Peanut digested into small allergenic fragments reaches the gut. (A) To induce tolerance, allergen fragments are sampled and captured by CX3CR1⁺ macrophages and transferred to CD11b⁺/CD103⁺ dendritic cells (DC). The activated DCs, producing IL-10 and retinoic acid, then migrate to the draining mesenteric lymph nodes where it presents the allergenic peptide to induce the differentiation of naïve CD4⁺ T cells to Foxp3⁺ inducible regulatory T cell (iTreg). Retinoic acid produced by the DCs will induce expression of $\alpha 4 \beta 7$ intergrin that help the survival and homing of iTreg to the gut to exert tolerance. (B) Desensitisation is initiated by a decreased in activation of effector cells (mast cells and basophils) and a population shift of TH2 to antigen-specific iTreg cells. Class-switching of allergen-specific B cells from IgE to IgG4 production which competes with IgE for binding to allergen will further down-regulation of TH2 cells mediated allergic response and thus decreasing allergic symptoms.

exposure to dose-dependent allergen decreases the reactivity of effector cells which recovers when administration was stopped. Desensitisation is mediated by the changes in the effector cells such as basophil and mast cells as shown in Figure 3(B). In addition, during OIT, antigen-specific CD4⁺ T cells were found to change from allergic and regulatory phenotypes to anergy and non-allergic phenotypes. Likewise, the allergen-specific B-cells undergo class-switch from allergen-specific-IgE to IgG4 production. Plasma from peanut-sensitive patients with detectable peanut-specific IgG4 was found to inhibit mast cell activation which was restored when the IgG4 was depleted indicating the role of IgG4 in inhibition of basophil and mast cells activation.³¹

Immunotherapy

Immunotherapy is using the body's immune system for treatment and has been the recent strategy to treat food allergy, including peanut allergy. Food allergen immunotherapy aims to establish functional immune tolerance or state of unresponsiveness instead of sensitisation leading to inappropriate inflammatory immune responses to the food allergen. Therefore, immunotherapy for peanut allergy attempts to desensitise the overactive immune response by repeated administration with increasing doses of the peanut or allergen in the absence of any danger signal, to achieve a state of sustained unresponsiveness where the probability of an allergic reaction decreases. The

exact mechanisms of desensitisation are not well understood and may differ from the healthy, pre-allergic state of immune tolerance. In a nutshell, a decrease in the IL-4 secreting T_H2 cells will down-regulate mast cells and basophils activation while IL-10 producing T_{reg} cells increases during immunotherapy.³²

Despite the high risk of peanut allergens-induced anaphylaxis, there exist several immunotherapy strategies such as OIT, subcutaneous immunotherapy (SCIT), sublingual immunotherapy (SLIT) and more recently the epicutaneous immunotherapy trials (EPIT). Repeated administration of a specific peanut allergen with increasing dosage has been shown to reduce sensitivity towards the allergen.⁵ OIT has been reported to successfully induce tolerance and desensitising children allergic to milk and eggs.^{33,34} SCIT involves the injection of a small dosage of an allergen under the skin while SLIT is the administration of small drops of allergen under the tongue.⁶ EPIT involve the use of patches or a specially developed delivery system of allergen application, both studies have shown EPIT are safe and effective for peanut allergy.^{35,36} OIT for peanut allergy is currently the most studied compared to SCIT, SLIT or EPIT and a number of studies on OIT are at phase I and phase II clinical trials.^{4,6}

During the initial administration of food-specific immunotherapy (either through OIT or SLIT), decrease in allergen-specific mast cell and basophil degranulation activity is observed early during the desensitisation process, resulting in the individual's ability to tolerate increasing amounts of the relevant food. Basophil reactivity diminished with peanut SLIT, as demonstrated by reduction in activated $CD63^+$ basophils after *in vitro* stimulation with peanut extract.³⁷ In addition, constitutive expression of $CD203c$ (a basophil activation marker) was reduced after SLIT and OIT for milk allergy.³⁸ Small amount of mediators (leukotriene and histamines) released below the threshold of systemic anaphylactic may shift the threshold activation of mast cells and basophils.³⁹ The reduction in allergen-specific-IgE levels and IgE-mediated skin sensitivity was observed much later, from as early as six months to two year of immunotherapy.⁴⁰ The concerted production of allergen-specific IgG4, reduction of T_H2 and IgE-producing cells and changes in cytokine profile of effector cells may further lead to the development of clinical desensitisation and possibly tolerance.^{40,41} Currently, the forefront of food allergy research revolves around deciphering the signalling pathways underlying

immune tolerance, sensitisation and desensitisation to food allergens.

Oral immunotherapy

OIT for allergic diseases may use "tolerising" vaccines containing the allergen or fragments of it.⁴² Allergen will be administered in the build-up phase with increasing amount regularly until the maximum dosage. The maintenance phase will begin once the effective therapeutic dose is reached, which involves daily administration of the allergen for a specific duration. However, the dosing regimens vary significantly between studies due to lack of standardisation of up-dosing protocols, maintenance doses and final doses for oral food challenge.²³ Similarly, peanut OIT attempts to desensitize the overactive immune response by repeated administration with increasing doses of peanut or its allergens.

Phase I trials of peanut OIT in subjects using whole crushed peanut, peanut flour or peanut powder have shown promising results. A multicentre US open label study reported successful desensitisation to peanut protein in 27 of 29 subjects with total peanut dose of 3.9 g and Blumchen et al. reported 14 of 23 (60%) peanut-allergic subjects reaching a maintenance dose of 500 mg of peanut after undergoing OIT with whole crushed peanut.⁴³ In addition, the first double-blinded placebo-controlled study of peanut OIT (using peanut flour as agent) was conducted where 16 out of 19 allergic children subjects were desensitised and able to reach the maximum cumulative dose of 5000-mg peanut protein challenge in a year duration of OIT treatment.⁴⁴ These subjects do not require epinephrine administration and the degree of protection was expected to prevent anaphylaxis due to accidental consumption of peanut. The largest phase II randomised-controlled crossover trial of peanut OIT was performed on 99 children, aged 7–16 years, having different severities of peanut allergy.⁴⁵ Two weekly increments up to a maximum dose of 800 mg peanut protein per day was administrated followed by a maintenance of 800 mg daily doses for 26 weeks. As a result, 84% of subjects were desensitised to 800 mg peanut protein whereas 62% were desensitised to 1400 mg peanut protein. OIT participants who successfully completed treatment exhibited lower levels of peanut-specific IgE at baseline were of younger age and lack family history of peanut allergy. In addition, they were able to eat large quantities of peanuts and showed a significant improvement in quality of life.^{45,46} Table 1 shows a summary of clinical studies

Table 1. Clinical studies using different dosing protocols for oral immunotherapy (OIT) of peanut allergy and the observed immunological changes.

OIT agent	Success subjects	Dosing protocol	Immunological change	References
Peanut flour	16 of 19	<i>Escalation:</i> 0.1–6mg, doubles every 30 min <i>Build-up:</i> 75 mg (25–33%) <i>Maintenance:</i> 4g, every 2 weeks, 44 weeks <i>Challenge:</i> 5g	↓SPT ↑ IL-5 and IL-13, peanut-specific IgG ₄ . ↑ then ↓ peanut-specific IgE ↑FoxP3 ^{hi} : FoxP3 ^{intermediate} CD4 + CD25 + T cells	[44]
Peanut flour	27 of 29	<i>Escalation:</i> 0.1–50mg, doubles every 30min <i>Build-up:</i> increased 25mg every 2 weeks until 300 mg <i>Maintenance:</i> 1800 mg daily <i>Challenge:</i> 3.9 g	↑ IL-10, IL-5, IFN- γ and TNF- α over 6–12 months ↓ Peanut-specific IgE by 12–18 months ↑ IgG ₄ Peanut-specific FoxP3 T cells ↑ 12 months then ↓	[42]
Whole crushed roasted peanut	14 of 23	<i>Escalation:</i> 0.0075–0.5 g doubles every 30 min ^a <i>Build-up:</i> 1 g on third day <i>Challenge:</i> 4g	↑ Peanut-specific IgG ₄ , ↓ IL-5, IL-4 and IL-2	[43]
Peanut flour	39 of 49	<i>Escalation:</i> 2, 5, 12.5, 25, 50, 100, 200, 400 and 800 mg <i>Maintenance:</i> 800 mg daily, 26 weeks <i>Challenge:</i> 1400mg	Small ↓ in median SPT weal diameter ↑ Peanut-specific IgE increase after 24 weeks OIT in the active group	[46]
Peanut flour	19 of 22 (6 weeks) 18 of 22 (30 weeks)	<i>Escalation:</i> 1, 5, 25, 50, 75 and 100 mg ^a <i>Build-up:</i> 0.5, 1, 2, 5, 12, 25, 50, 100, 200, 400 and 800 mg for 2 weeks <i>Maintenance:</i> 800 mg daily, 30 weeks <i>Challenge:</i> 2.6 or 6.6g (6 or 30 weeks)	↓ SPT at 6 ug/mL after 30 weeks ↑ Peanut-specific IgE then ↓ at 30 weeks ↓ Serum Ara h 2-IgE when challenged after 30 weeks	[45]
Peanut powder	15 of 22	<i>Escalation:</i> 33, 66, 133, 199 and 364 mg, every 30 min ^a <i>Up-dosing:</i> 16–795mg (9–55%) <i>Maintenance:</i> 795 mg daily, 3 months <i>Challenge:</i> 795 mg	↑ Median Ara h2-specific IgE at first month but ↓ significantly at >6 months ↑ Peanut-specific IgG ₄ levels and Ara h 2-specific IgG ₄ after 1 month	[77]
<i>Lactobacillus rhamnosus</i> CGMCC1.3724	23 of 31	<i>Escalating:</i> 2 × 10 ¹⁰ CFU (freeze-dried powder) daily together with peanut OIT daily for 18 months <i>Build-up:</i> 2g every 2 weeks, 8 months <i>Maintenance:</i> 2g every 2 weeks, 10 months <i>Challenge:</i> 4g	↓ Peanut-specific IgE levels ↑ Peanut-specific IgG ₄ levels	[73]

^aDBPCFC = Double-blind placebo-controlled food challenge.

for OIT of peanut allergy and the immunological changes observed. Most of the protocols include escalation in amount of peanut (e.g. peanut flour or peanut powder) administered followed by maintenance at a prolonged period and then challenged to determine the effectiveness of OIT to induce desensitisation or tolerance.

Approximately 30–40% peanut-allergic patients are also allergic to other nuts.⁴ Simultaneous administration of multiple food allergens (of peanut with tree nuts, milk, egg or sesame) showed that participants were able to achieve a 10-folds increase from the initial challenge threshold and only two subjects had severe reactions requiring epinephrine. This study concluded that OIT can safely desensitise up to a combination of five allergens and is therefore, a potential treatment for multiple food allergies as it is time and cost saving.⁴⁷

Concerns for existing oral immunotherapy

OIT for peanut allergy treatment has been very promising based on past results. Although no casualty has been associated with OIT protocols, despite

encouraging results, it is still limited to clinical trial settings as its long-term effects require further studies. Despite being generally safe, it is not without risk as some subjects were unable to endure the effects associated with peanut OIT and thus it may not be suitable for all patients.⁴⁸ Although a Cochrane systematic review concluded that the peanut OIT is a promising therapeutic approach, there is still not enough evidence to draw a conclusion on its safety, cost and long-term effectiveness. In fact, an online survey on a total of 442 clinicians indicated that 13.8% clinicians were either doing clinical trial or started providing OIT.⁴⁹

Acceptance of OIT as a treatment for peanut allergy by the regulatory agencies will require a standardised and well-characterised product which can be reproducibly manufactured. In addition, for OIT to be used in routine clinical practice, approved clinical trials procedures and a suitable risk-benefit profile is necessary. Recently, an oral biological product comprising of defatted peanut, AR101 has been standardised for pharmaceutical application and shown to suppress allergic reaction after two weeks of administration of 300 mg of peanut protein in a phase 2 clinical trial for OIT of peanut-allergic children and

young adults.⁵⁰⁻⁵¹ In addition, after maintenance with AR101 for six months, tolerance to peanut protein increased up to 18-fold, although larger study population is needed to better understand the safety and efficacy of AR101.

Although OIT shows clinical efficacy on desensitisation and are more often evaluated compared to SLIT and EPIT, the later may be better in terms of safety compared to OIT.^{52,53} SLIT has a good safety profile especially for inhalant allergy, inducing lower amount of effector cells (thus lower risk of allergic reaction) but abundant antigen-presenting dendritic cells which promote the induction of tolerogenic T_{reg} cells in the sublingual mucosa.^{54,55} Peanut allergen applied on the skin in EPIT has shown no systemic reaction, only common local eczematous skin reactions.⁵⁶ This may be because the skin epidermal layer is not well vascularised and contain high amount of antigen-presenting cells with immune modulation ability, thus limiting the systemic reaction. The better safety profile of SLIT and EPIT may appear to surpass OIT; however the lower doses use in SLIT and EPIT may limit the efficacy.^{57,58} In addition, the efficacy of EPIT and SLIT is comparable due to low dosage use, but the small increment in reactive dose may not be sufficient to provide protection in the case of true accidental exposures.^{38,59} More research and systematic review are needed to outline the risk of these different routes of immunotherapy and to standardise the regiment, dosage and desensitisation strategy.

Recombinant peanut allergen for oral immunotherapy

Numerous studies have shown OIT using peanut protein as a promising treatment for peanut allergy. However, one drawback in using peanut protein is the lack of standardisation in the amount of protein administered. Thus to address this issue, the use of recombinant peanut allergen offers a promising strategy using component-resolved treatment in OIT for peanut allergy.

A total of 17 peanut allergens has been identified and recognised by the WHO/IUIS Allergen Nomenclature Sub-committee. They were characterised based on their biological function and placed in protein superfamilies such as the Cupin, Prolamin, Bet v-1 like, Profilin Glycosyl transferase GT-C, Scorpion toxin-like knottin and the recently reported lipid transfer proteins as shown in Table 2.⁶⁰ Among these allergens, Ara h 1, Ara h 2 and Ara h 3 are classified as the major peanut allergens due to their ability

Table 2. Comparison of peanut allergens from cupins, prolamins, Bet v1, profilin, oleosin, defensin and transfer protein families and their biological function and binding to IgE

Protein Superfamily	Protein Family	Allergen	Size; Isoelectric point	Prevalence of IgE binding (%)
Cupin	Vicillin or 7s globulin Legumin or 11s globulin	Ara h 1	65.3kDa; pI= 4.6 (Trimer of 180 kDa)	30–80
		Ara h 3	60.0kDa; pI= 4.6 (hexamer of 360 kDa)	16–57
Prolamin	2S albumin	Ara h 2	16.6kDa; pI= 5.8; 18.0kDa; pI= 5.5 (dimer)	42–100
		Ara h 6	15.0kDa; pI= 7.5	86–92
Bet v 1-like Profilin Glycosyltransferase GT-C	nslTP Bet v 1 family Profilin Oleosin	Ara h 7	16.4 kDa; pI= 5.6; 17.4kDa; pI= 7.5	43
		Ara h 9	9.1kDa; pI = 9.3	8–60
		Ara h 8	17.0kDa; pI = 5.0	22–66
		Ara h 5	14.0kDa; pI= 4.6	3–24 birch or grass pollen allergy
		Ara h 10	17.6kDa; pI = 9.6	not known
		Ara h 11	14.3kDa; pI = 10.1	
		Ara h 14	17.5kDa	
		Ara h 15	17kDa	
		Ara h 12	5.2kDa; pI = 7.7	not known
		Ara h 13	8.4kDa; pI = 7.5	not known
Scorpion toxin like knottin	Plant defensin	Ara h 16	8.5kDa (SDS-PAGE reducing)	
Non-specific lipid transfer protein	Transfer protein	Ara h 17	11kDa (SDS-PAGE reducing)	

to recognise IgE in more than 50% of peanut-allergic patients.⁶¹ Ara h 6 was reported to exhibit similar allergenic potential and activity as Ara h 2.⁶²

The recombinant allergens approach also enables the development of hypoallergenic allergens which are deemed safer for OIT application. DNA sequence of IgE epitopes can be altered by site-directed mutagenesis to reduce reactivity to IgE and thus providing pure and standardised allergens as an alternative approach for immunotherapy, with reduced side effects.^{63,64} Amongst the major peanut allergens, Ara h 2 is the preferred diagnostic and therapeutic reagent because it reacts to specific IgE in more than 90% of peanut-allergic patients.⁶²

Production of recombinant Ara h 2 and Ara h 2.01 in *Escherichia coli* and *Lactococcus lactis* has been previously reported, respectively.^{65–67} Recently, we reported the use of a codon-optimized Ara h 2.02 in pET-28a (+) expression system for recombinant protein production in *E. coli*.⁶⁸ Ara h 2.02 compared to Ara h 2.01 is the more potent of the two isoforms of Ara h 2 due to an additional IgE epitope that confers higher binding and therefore a more potent cross-linker of IgE. We further demonstrated that the recombinant Ara h 2.02 (rAra h2.02) allergen produced in *E. coli* was able to elicit a comparable level of allergen-specific IgE in sensitised *Balb/c* mice. In addition, the specific-IgE antibodies raised against rAra h2.02 were able to recognise the native Ara h2 protein, demonstrating its allergenicity and potential for diagnosis and therapeutic study.

Probiotics in the treatment of allergies

Probiotics are live microorganisms capable of conferring health benefits on the host when administered in adequate amounts.^{69,70} Commercially available probiotics include the *Lactobacillus* sp. (*L. acidophilus*, *L. casei*, *L. plantarum*), *Bifidobacteria* sp. (*B. bifidum*, *B. bifidum*, *B. animalis*) and *Lactococcus* sp. (*L. lactis*, *L. subtilis*). Probiotics contribute to the maintenance of intestinal microbial balance and modulate the host's immune system. They have been shown to promote hypoallergenic effect, protecting against allergy.⁷¹ *Lactococcus* sp. and *Lactobacillus* sp. are food-grade lactic acid bacteria (LAB) and “generally recognised as safe” (GRAS) bacteria. Therefore, these can be a preferred choice as mucosal delivery vehicles for vaccine approaches. A study on *L. plantarum* has shown its ability to elicit immunomodulatory effects on the T_H2 cells responses in allergic disorders.⁷² We previously demonstrated that *L. casei* shirota strain has the ability

to suppress T_H2 activation and proinflammatory cytokines production in a dustmite allergy mouse model.⁷¹ More recently, the first randomised trial combining probiotic with peanut OIT was shown to be highly effective in modulating the peanut-specific immune response in seven out of nine children treated and showing sustained unresponsiveness.⁷³

There are few proposed mechanisms for the action of probiotics in preventing or down-regulating allergy responses. Probiotics can prevent inflammatory progression by reducing the intestinal microenvironment and the permeability barricade of the gut, thus changing their immunogenicity.⁷⁴ Besides this, they also act as immune response modulator by altering the cytokine profiles down-regulating T_H2 cytokines and up-regulating T_H1 cytokines production, redirecting the immune system to a regulatory mode. The ability to stimulate the production of IFN- γ , TNF- α and IL-12 helped to regulate towards a T_H1 response, concurrently inhibits the production of allergen-specific IgE. Probiotics can also modulate the toll-like receptors (TLRs) which lead to the activation of dendritic cells, inducing T_H1 response while suppressing T_H2 responses.⁷⁴

Recombinant probiotics for OIT

For OIT, probiotics can be a potential carrier to facilitate the oral delivery of *in vivo* expressed peanut derivatives. Administration of heat-killed *Listeria monocytogenes* (hkLM) expressing Ara h 2 was found to induce potent immune response in mouse due to activation of the innate immune signalling by the bacterial product.⁷⁵ The peanut-allergic mice model that were treated with a mixture of the three major peanut allergens (Ara h 1, 2 and 3) and hkLM showed significant reduction in peanut allergen-specific IgE and were protected against systemic anaphylaxis. This study shows that the protective effect was due to the down-regulation of T_H2 responses while T_H1 responses were up-regulated. Another study reported the use of *Streptococcus thermophilus* expressing recombinant Bet v1 (major allergen of *Betula verrucosa*) as a delivery tool for OIT of Bet v1-sensitised mice.⁷⁶ These mice showed specific local and systemic anti-inflammatory responses and the clearance of lung eosinophilia thus indicating that the allergen was delivered in an immunologically active form to stimulate the resident immune cells to elicit immunomodulatory effects.

High-yield expression and purification of secreted Ara h 2 using pAMJ399 expression vector (a “self-

induced" vector at pH 6.5 and below) in *L. lactis* has been reported but to date, there is no report on its use for immunotherapy.⁶⁶ The potential of engineered probiotics producing recombinant peanut allergen for OIT is currently being explored for their ability to down-regulate the allergy reaction owing to the inherent property of probiotic and ability to induce tolerance or desensitisation to the delivered allergens.⁷⁴

Conclusion

In conclusion, the use of OIT in treating peanut allergy shows promising future based on the clinical trials and published results, despite issues related to regiment strategy, the variation caused by lack of standardisation on dosage and timeline for the treatment. The production and purification of recombinant allergen are cost and time effective compared to crude extract. In addition, consistent quality with minimal batch-to-batch variation and higher purity of recombinant allergens enables its use as component resolved treatment in OIT for peanut allergy. The combination of recombinant allergen with probiotic as a carrier offers a promising immunotherapy strategy which is safe and leveraging on the immunomodulation properties of probiotics to enhance tolerance or desensitisation to peanut allergy.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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