

REVIEW ARTICLE

Oral immunotherapy and tolerance induction in childhoodM. L. K. Tang^{1,2,3} & D. J. Martino⁴¹Allergy and Immune Disorders, Murdoch Childrens Research Institute, Melbourne, Vic., Australia; ²Department of Allergy and Immunology, Royal Children's Hospital, Melbourne, Vic., Australia; ³Department of Paediatrics, University of Melbourne, Melbourne, Vic., Australia;⁴Gastroenterology and Food Allergy, Murdoch Childrens Research Institute, Melbourne, Vic., Australia**To cite this article:** Tang MLK, Martino DJ. Oral immunotherapy and tolerance induction in childhood. *Pediatr Allergy Immunol* 2013; **00**.**Keywords**

childhood; desensitization; food allergy; oral immunotherapy; oral tolerance

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Accepted for publication 31 May 2013

DOI:10.1111/pai.12100

Abstract

Prevalence rates of food allergy have increased rapidly in recent decades. Of concern, rates of increase are greatest among children under 5 yrs of age and for those food allergies that persist into adulthood such as peanut or tree nut allergy and shellfish allergy. Given these trends, the overall prevalence of food allergy will compound over time as the number of children affected by food allergy soars and a greater proportion of food-allergic children are left with persistent disease into adulthood. It is therefore vital to identify novel curative treatment approaches for food allergy. Acquisition of oral tolerance to the diverse array of ingested food antigens and intestinal microbiota is an active immunologic process that is successfully established in the majority of individuals. In subjects who develop food allergy, there is a failure or loss of oral tolerance acquisition to a limited number of food allergens. Oral immunotherapy (OIT) offers a promising approach to induce specific oral tolerance to selected food allergens and represents a potential strategy for long-term curative treatment of food allergy. This review will summarize the current understanding of oral tolerance and clinical trials of OIT for the treatment of food allergy.

Prevalence rates of food allergy and food anaphylaxis have increased exponentially in recent decades, particularly in countries with a Westernized lifestyle. In an Australian study, the increased prevalence of food anaphylaxis over the past decade was shown to primarily affect children under 5 yrs of age, and rates of increase were greatest for peanut and shellfish allergies (approximately 300% increase between 1994 and 1998) (1). This means that rates of food allergy will likely compound as the food allergies that are rising most rapidly are those that will persist into adulthood. Food allergy develops as a result of failure or loss of oral tolerance. Novel therapeutic approaches that can establish or restore oral tolerance to food allergens would offer a long-term curative treatment for food allergy.

Oral tolerance

Oral tolerance refers to antigen-specific tolerance induced in the periphery within the gut-associated lymphoid tissues (GALT). Wells and Osborne first described oral tolerance more than a century ago in 1911, having observed that guinea pigs failed to develop anaphylaxis to previously ingested dietary antigens (2). Many decades later, it was shown that oral tolerance is an active immunologic process and not merely the absence of an immune response to orally administered antigens. Adoptive transfer of CD4⁺ or CD8⁺ T cells

harvested from mice previously tolerized to an antigen through oral feeding could confer antigen-specific tolerance in recipient naïve mice (3, 4).

In rodent models, oral tolerance can be induced either by ingestion of a single high dose of antigen (5–100 mg) or by repeated ingestion of low doses of antigen (0.5–1.0 mg/day for 5 days) (5, 6). High-dose antigen ingestion induces anergy or clonal deletion of antigen-specific T-helper cells. T-cell anergy describes a process whereby antigen-specific T-cells are functionally arrested when antigen presentation occurs in the absence of costimulatory molecules (5). Anergized T-cells persist in the periphery, but lose their capacity to clonally expand and to synthesize the full repertoire of cytokines following antigen encounter, instead being restricted to the production of IL-10. In OVA-fed transgenic mice, both Th1 and Th2 cells underwent deletion following their initial activation by high-dose antigen exposure, while TGF-secreting T regulatory cells (Treg) were resistant to deletion (6). This suggests that Treg may also play a role in high-dose tolerance.

Repeated low-dose ingestion of antigen leads to induction of antigen-specific T regulatory cells that have suppressor activity. Various T regulatory cell populations may be induced including TGFβ-producing CD4⁺ T helper-3 cells (Th3) (4) and gut-derived antigen-specific CD4⁺ CD25⁺ Foxp3⁺ T cells known as induced T reg (iTreg) (7). While various T-cell subsets with regulatory activity can confer tolerance in transfer

experiments, it has been shown that gut-derived iTreg are critical for oral tolerance induction, with deletion of iTreg in a DEREK mouse model resulting in loss of tolerance (8). In contrast, naturally occurring thymus derived Treg (nTreg) do not appear to be required for successful oral tolerance induction (9). Also, oral tolerance can develop in CD8 knockout mice (10) suggesting that the role of CD8⁺ T cells may be restricted to maintenance as opposed to induction of oral tolerance.

Generation of iTreg and tolerance induction within the gut-associated lymphoid tissues

The GALT is responsible for maintaining mucosal immune homeostasis; that is, discriminating between pathogenic and innocuous antigens within the intestinal lumen and invoking immunity to pathogens while inducing and maintaining tolerance to innocuous antigens. Generation of Treg, particularly iTreg, is fundamental to the induction and maintenance of oral tolerance. This process is directed by specialized dendritic cells (DC), which exist as two distinct subsets within the

intestine – CD103⁺ DC that are capable of migration to mesenteric lymph nodes (MLN), and CX3CR1⁺ DC that remain resident within the gut (11, 12). Digested dietary peptides can be presented to intestinal DC in a number of ways (Fig. 1). Luminal antigens can be sampled by specialized M-cells in the follicular epithelium overlying Peyer's patches, with subsequent presentation to CD103⁺ DC. Alternatively, CX3CR1⁺ DC may directly sample luminal antigens by extending dendrites between epithelial cells (13). Luminal antigens that cross the intestinal epithelium may also be processed by CD103⁺ DC. A critical next step involves the CCR7-dependent migration of CD103⁺ DC to the MLNs (14). In the MLNs, tolerogenic CD103⁺ DC induce differentiation of naïve T cells into CD4⁺ CD25⁺ Foxp3⁺ induced Treg (iTreg) and imprint gut-homing properties on these iTreg (9). This process requires retinoic acid (RA) and appears to be positively influenced by indoleamine-2,3-dioxygenase, B7 family of costimulatory molecules and TGF- β (9, 15, 16). These iTreg generated within the MLN migrate back to the intestinal lamina propria where interaction with resident CX3CR1⁺ DC results in expansion of antigen-specific iTreg, allowing sup-

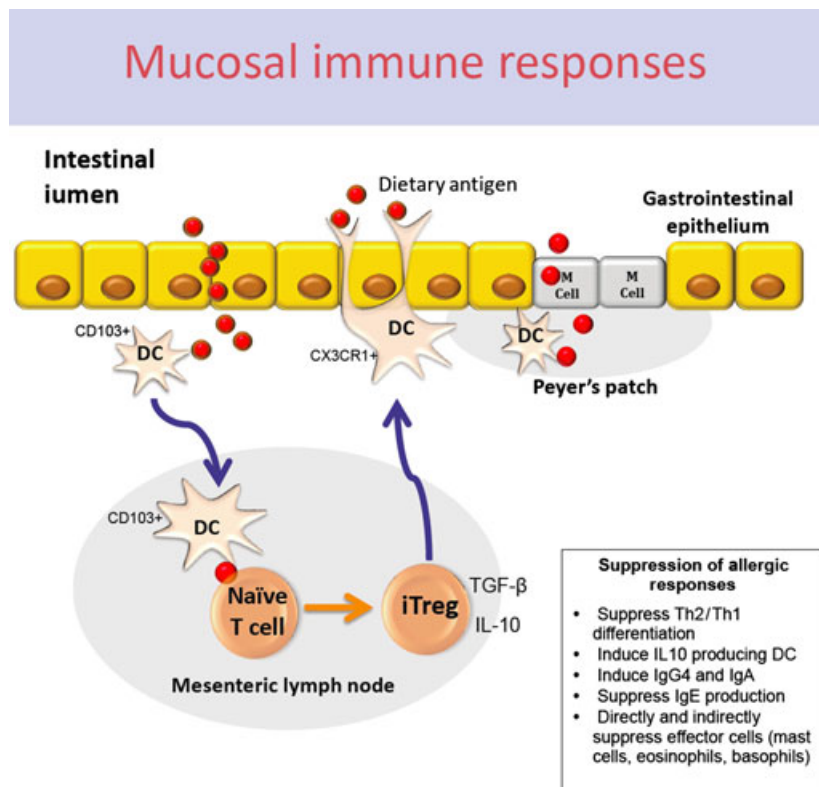


Figure 1 Mucosal immune responses and the generation of iTreg. The mucosal immune response to orally ingested antigens generally results in oral tolerance. Gut-derived induced Treg (iTreg) play a crucial role in this process. The generation of iTreg is directed by specialized dendritic cells (DC) resident in the gut. Antigens within the intestinal lumen are sampled in a number of ways – specialized M-cells overlying the Peyer's patches can sample antigen for presentation to underlying CD103⁺ DC, CX3CR1⁺ DC can directly sample antigens by extending dendrites between intestinal epithelial cells, and antigen may cross the epithelium (via epithelial cell uptake or direct passage across the epithelium) to be processed by CD103⁺ DC within the lamina propria. CD103⁺ DC migrate to mesenteric lymph nodes (MLN) where they interact with naïve T cells, inducing differentiation to CD4⁺ CD25⁺ Foxp3⁺ induced Treg (iTreg). iTreg generated in the MLN migrate back to the intestine, where encounter with CX3CR1⁺ DC promotes their expansion.

pression of antigen-specific immune responses locally in the gut (8). While iTreg play a critical role in oral tolerance, other major classes of Treg are also induced following oral ingestion of antigen and can contribute to regulatory effects, including TGF β -secreting (LAP⁺) Th3 cells, IL10-secreting Tr1 cells, and CD8⁺ Treg (17).

Food allergy: a manifestation of failure or loss of oral tolerance

Food allergy is suggested to occur as a result of failure or loss of oral tolerance. Studies of airway allergy indicate that allergen-specific Th1, Th2, and Treg cells can be identified in both allergic and non-allergic individuals, and it is the balance between allergen-specific Treg and Th2 cells that provides the critical factor which determines progression to allergy vs. tolerance (18). Aeroallergen-allergic subjects were shown to have a predominance of allergen-specific Th2 responses with relative deficiency of allergen-specific Treg responses, while non-allergic subjects displayed a dominant Treg response or an attenuated response to allergens (18). A similar paradigm appears to be present in food allergy. IgE-mediated food allergy is associated with production of allergen-specific IgE, low or no allergen-specific IgG1 or IgG4, as well as increased Th2 and low Treg cytokine responses to allergen (19–23), whereas non-allergic children demonstrate a Treg or Th1 predominant cytokine response (21–23) or fail to respond to allergen (20, 22). In addition, children with food allergy have fewer TGF β ⁺ lymphocytes in duodenal epithelium and lamina propria as well as reduced TGF β expression in duodenal epithelium (19, 24). Moreover, this reduced T regulatory activity in children with food allergy is present from the time of birth, prior to the onset of disease (25). Conversely, resolution of food allergy was associated with reduction in allergen-specific IgE and reversion to Th1 dominant cytokine responses to allergen (21). In addition, patients who had outgrown their cow's milk allergy had increased numbers of circulating CD4⁺ CD25⁺ cells (presumed Treg) and reduced proliferative responses to β -lactoglobulin compared to those with persistent cow's milk allergy (26). Depletion of CD4⁺ CD25⁺ cells in peripheral blood mononuclear cell cultures from children who had acquired tolerance resulted in a marked increase in β -lactoglobulin-induced proliferative responses *in vitro*, suggesting that resolution of food allergy is associated with increased allergen-specific Treg activity (26). Studies of individual T-cell populations using tetramer technology indicate that allergic patients also exhibit a higher frequency of allergen-reactive cells compared with non-allergic individuals (27).

Nevertheless, some studies have not supported these findings. Th2 cytokine production was reported in both adults with food allergy as well as those who were tolerant, with or without sensitization to the food, although the intensity of Th2 cytokine production was highest in those with food allergy (28). Children with cow's milk allergy had increased FoxP3 mRNA expression in β -lactoglobulin-stimulated peripheral blood mononuclear cells compared to either non-allergic controls or those with spontaneously resolved cow's milk

allergy, and spontaneous resolution of cow's milk allergy was not associated with Treg activation (29). A similar frequency of allergen-specific CD25⁺ CD27⁺ Treg was observed in cow's milk-allergic and non-allergic subjects, although cow's milk-allergic subjects who were tolerant to heated milk (which may represent a milder phenotype of cow's milk allergy) had a higher frequency of CD25⁺ CD27⁺ regulatory T cells compared with those allergic to both raw and cooked forms of cow's milk (30). These inconsistent findings for Treg activity in food allergy may relate to differences in clinical parameters (e.g., age of study population, time since exposure to food allergen) and/or technical differences in Treg assays. While Treg populations are known to play a critical role in the induction of oral tolerance, further studies are required to better elucidate their role in the pathogenesis of food allergy.

Searching for an effective treatment: desensitization vs. tolerance

The search for effective long-term treatments for food allergy has focused on strategies that can induce oral tolerance, with oral immunotherapy (OIT) receiving greatest interest. In evaluating the effectiveness of potential definitive therapies, it is important to understand the distinction between 'desensitization' and 'tolerance'. Desensitization describes the ability to ingest a food without reaction while continuing to ingest regular doses of that food (e.g., continuing on OIT). This state of desensitization is mediated by changes in effector cells (mast cells, basophils), without modulation of underlying pathogenic immune mechanisms (31). Hence, the individual remains allergic to the food allergen and ingestion of the food after a period of elimination (discontinuation of immunotherapy) would result in an acute allergic reaction. In this respect, desensitization to a food is similar to the process of drug desensitization in drug allergy. In contrast, tolerance is the ability to tolerate a food after a period of time has elapsed since ingesting the food (e.g., after discontinuation of OIT). The acquisition of tolerance is believed to reflect reprogramming of the immune response to allergen, involving regulatory T cells or other T-cell subsets and/or allergen-specific anergy and clonal deletion. Tolerance is expected to persist for at least months or years after the food therapy has been discontinued (31). Induction of long-lasting allergen-specific tolerance has been demonstrated in the setting of subcutaneous immunotherapy for insect sting allergy and aeroallergen allergy. In the context of food allergy, desensitization and tolerance can be determined by performing food challenges. Desensitization is demonstrated by performing oral food challenge while a subject is still receiving treatment (such as OIT) or eating regular doses of a food, whereas tolerance is confirmed by performing oral food challenge after discontinuing food ingestion for a period of at least 2–4 wks (31).

Oral immunotherapy for food allergy

Oral immunotherapy was first described in 1908 in a patient with egg anaphylaxis (32). Almost a century later, case reports of OIT to cow's milk revealed effective desensitization (33, 34),

associated with immune changes including reduced cow's milk IgE and cow's milk-induced IL4 production, together with increased cow's milk IgG4 and IgA as well as cow's milk-induced IFN γ production (34). These early reports led researchers to pursue OIT as a promising approach to the treatment of food allergy, and numerous case series, pilot studies, and randomized controlled trials (RCT) have since been completed [reviewed in (35)].

Randomized controlled trials of cow's milk, egg, and peanut OIT have consistently reported complete desensitization (passed food challenge while on OIT) in 33–90% of subjects (36–42). A recent Cochrane meta-analysis of cow's milk OIT trials confirmed the beneficial effects of cow's milk OIT in inducing desensitization when compared to a standard cow's milk elimination diet (43). It should be noted that the Cochrane review used the term 'total tolerance' to denote desensitization as all included studies assessed for desensitization and not tolerance as defined by the NIAID workshop on food allergy clinical trials (31).

In contrast to the large number of studies evaluating the ability for OIT to induce desensitization, few OIT studies have assessed for tolerance as a study outcome (Table 1). Six studies have presented data on tolerance following OIT – 3 were uncontrolled open studies, three were RCT. The first study to evaluate tolerance as an outcome for OIT was a small pilot study of egg OIT that reported tolerance in 2 of 7 (29%) subjects (44).

In a follow-up study, the same investigators applied a modified OIT protocol that adjusted the dose and duration of treatment according to the subject's egg sIgE – doses were escalated (to a maximum of 3.6 g egg protein) if sIgE remained >2 kU/l and maintenance treatment was continued until egg sIgE fell below 2 kU/l at which time food challenges were performed to assess for desensitization and tolerance. Six children reached the end of study (after 18–50 months of treatment) and all six achieved tolerance (45). As there were no control subjects in these studies, it is difficult to ascertain whether the rate of tolerance associated with OIT would have been greater than the rate of spontaneous resolution of egg allergy. A third open study of peanut OIT in 23 children with peanut allergy (confirmed by DBPCFC at study entry) reported tolerance in 17% of subjects (4/23) (46). Acquisition of tolerance in these subjects was likely to be associated with OIT since DBPCFC was performed at study entry; however, there remains the possibility that a similar rate of spontaneous resolution of allergy might be observed in a control group. Therefore, it is difficult to discern the effectiveness of OIT to induce tolerance from these studies.

Results from three RCT are also available. Staden et al. (47) randomized 45 children with egg or milk allergy to receive OIT (maximum dose 2.8 g egg protein or 8.25 g cow's milk protein) or an elimination diet for 18–24 months. At the end of the study, DBPCFC was performed 2 months after a secondary strict elimination diet to assess for tolerance. Tolerance was

Table 1 Oral immunotherapy (OIT) studies that have assessed for tolerance as an outcome

	Study design sample size	Allergen	Duration OIT	Rate of tolerance
Buchanan et al. 2007	Open label n = 7	Egg (300 mg egg powder)	2 yrs	2/7 (29%)
Vickery et al. 2010	Open-label study n = 6	Egg (3.6 g egg protein)	Median 33 months (18–50 months)	6/6 (100%)
Blumchen et al. 2010	Open-label NB: DBPCFC at study entry n = 23	Peanut (125–500 mg peanut)	2–22 months	4/23 (17%)
Staden et al. 2007	RCT – open-label OIT (n = 25) vs. avoidance (n = 20) NB: DBPCFC at study entry	Cow's milk (8.2 g CM Protein); Egg (2.8 g egg protein)	Median 21 months (11–59 months)	9/25 (36%) OIT 7.20 (35%) placebo
Burks et al. 2012	RCT – hybrid placebo controlled/open-label* OIT (n = 40) vs. placebo (n = 15)	Egg (2 g egg white solid)	22 months*	11/40 (28%) OIT 0/1 (0%) control
Keet et al. 2012	RCT – open label SLIT (n = 10) vs. SLIT/OIT 1 g (n = 10) vs. SLIT/OIT 2 g (n = 10)	Cow's milk SLIT (7 mg CM protein) SLIT/OIT (1 g CM protein) SLIT/OIT (2 g CM protein)	SLIT median 58 wks (56–62 wks) SLIT/OIT median 76 wks (68–89 wks)	1/10 (10%) SLIT 3/10 (30%) SLIT/OIT 1 g 5/10 (50%) SLIT/OIT 2 g

RCT, randomized controlled trials.

*Subjects were unblinded at 10 months after which time the study was continued as an open-label RCT with active participants remaining on OIT while placebo treatments were discontinued in control subjects. At 22 months, desensitization was assessed by DBPCFC in all active group participants (n = 34) and any control group participants with egg sIgE <2 kU/l (n = 1). Subjects that passed this 22 month DBPCFC progressed to a further DBPCFC at 24 months after a secondary elimination diet (discontinuing OIT) for 4–6 wks to assess for tolerance as defined by the NIAID workshop on Food Allergy Clinical trials (31). All subjects in the OIT group who failed to acquire tolerance at 24 months were recommenced on OIT, which will be continued for up to two additional years.

demonstrated in 36% of subjects within the active group and 35% of subjects in the control group, indicating that the OIT intervention was no more effective than elimination diet in the induction of tolerance (47). In another RCT of egg OIT, Burks et al. (48) randomized 55 children with egg allergy to receive egg OIT ($n = 40$) or placebo treatment ($n = 15$). Participants and investigators were unblinded at 10 months, after which time the trial was continued as an open study. At 22 months, a DBPCFC was performed in all subjects within the active treatment group ($n = 30$) and those placebo group participants with egg sIgE < 2 kU/l ($n = 1$) to assess for desensitization. The investigators elected to only challenge placebo subjects if egg sIgE < 2 kU/l as levels above this were previously shown to be associated with risks during food challenge and an increased likelihood of allergy. Subjects who passed the 22 month DBPCFC progressed to a second DBPCFC 4–6 wks after a secondary elimination diet (discontinuation of OIT in the active group) to assess for tolerance. Eleven of 40 subjects (28%) in the active OIT group and none (0/1) of the subjects in the placebo group acquired tolerance. While these results appear encouraging, it is important to consider the potential bias introduced by only performing DBPCFC to assess for tolerance in a selected subgroup of participants within the placebo arm. It is possible that a proportion of placebo subjects with egg sIgE > 2 kU/l may have outgrown their egg allergy within a 2-yr period. Indeed, many of the subjects in the control arm of the Staden study who spontaneously developed tolerance were reported to have sIgE > 2 kU/l (47). Moreover, in the Staden et al. study (47), 35% of subjects within the control arm outgrow their egg or cow's milk allergy by the end of study (median 21 months; range 11–59 months), which is similar to the rate of tolerance reported for subjects receiving OIT in the Burks et al. study (48).

A third open-label RCT randomized 30 subjects with cow's milk allergy to receive SLIT at a maintenance dose of 7 mg milk protein, SLIT followed by OIT at a maintenance dose of 1 g milk protein or SLIT followed by OIT at a maintenance dose of 2 g milk protein ($n = 10$ per group) (49). All subjects were commenced on SLIT (3.7 mg milk protein) prior to being randomized to one of 3 treatment groups. After maintenance dosing for 48 wks, an open cow's milk challenge was performed to assess for desensitization. Those that passed this challenge discontinued treatment and were rechallenged 1 and 6 wks later – subjects that passed the challenge 6 wks after cessation of treatment were considered to have acquired tolerance. One of 10 subjects who received SLIT alone, 3 of 10 subjects who received SLIT/OIT 1 g milk protein, and 5 of 10 subjects who received SLIT OIT 2 g milk protein achieved tolerance (no significant differences between groups). However, when all subjects receiving SLIT/OIT were pooled, the rate of tolerance with SLIT/OIT tended to be higher than the rate of tolerance with SLIT alone ($p = 0.09$). These findings should be interpreted with caution since the sample size is small and no placebo group was included. Nevertheless, these findings are encouraging, with SLIT/OIT appearing more effective than SLIT alone for induction of tolerance.

A recent meta-analysis aimed to evaluate the effectiveness of OIT in inducing tolerance (reducing the risk of allergy);

however, only one of the three RCT included in the analysis assessed for tolerance as an outcome, with the other two RCT only assessing for desensitization (50). Data from all three studies were subjected to a pooled analysis, and the authors concluded that OIT was not effective for the induction of tolerance. Given that only one of three studies evaluated for tolerance it is not possible to draw any conclusions from the findings. In summary, based on the current evidence, OIT is effective for the induction of desensitization; however, the ability for OIT to induce long-lasting tolerance remains uncertain. Future OIT studies should evaluate for tolerance as an outcome and include placebo and active groups to control for natural resolution of allergy, particularly for studies of cow's milk and egg allergy which are associated with high rates of spontaneous resolution (36, 47). Meta-analyses of OIT studies evaluating tolerance as an outcome are warranted once additional RCT are completed.

Immunologic changes associated with OIT

Although the ability for OIT to induce clinical tolerance remains uncertain, OIT has been shown to modulate allergen-specific immune responses, directing them toward responses typically associated with tolerance. Importantly however, all of the observed immune changes have been associated with both desensitization and tolerance following OIT, suggesting that the immune effects identified to date are not, of their own accord, critical determinants of tolerance acquisition.

Oral immunotherapy is most commonly associated with a reduction in allergen-specific IgE and an increase in allergen-specific IgG4. An increase in serum levels of sIgG4 has been universally reported (when assessed) (44–46, 48, 51), whereas a reduction in sIgE levels has only been noted in some studies (41, 45, 47, 51–53) with others reporting no change in sIgE levels (40, 42, 44, 48, 54). The increase in allergen-specific IgG4 was shown to reflect a broadly expanded IgG4 clonal repertoire with novel specificities to allergen components, while reduced levels of peanut-specific IgE were associated with reduced diversity in a subset of individuals (55). Interestingly, Burks et al. (48) reported that subjects receiving egg OIT developed reduced egg SPT as compared to placebo treated subjects, despite no differences in serum levels of egg sIgE between the two groups. This reduction in SPT reactivity presumably resulted from inhibition of basophil (and mast cell) activation and/or increased peanut sIgG4 levels (48) that were associated with OIT. Indeed, IgG4 has been shown to inhibit formation of IgE immune complexes and hence basophil activation. In this same study, reduced basophil activation was associated with desensitization, while increased production of IgG4 antibody and a reduction in skin prick test wheal size relative to baseline were linked to oral tolerance, suggesting the latter may be more relevant markers of tolerance (48). The mechanisms mediating the shift in allergen-specific antibody isotype from sIgE to sIgG4 during OIT (in the setting of both desensitization and tolerance) are unclear. There is some evidence this could involve downregulation of T-cell apoptotic pathways (51) or clonal deletion of allergen-specific IgE-producing B cells

together with clonal expansion of allergen-specific IgG4-producing B cells (55).

Changes in cytokine responses have been reported with OIT. Reduced Th2 and increased Treg responses are common findings. Peanut OIT was associated with reduced allergen-induced Th2 cytokine production (37, 46). Blumchen et al (46) found that desensitization was associated with reduced IL5 production, while sustained tolerance was associated with a reduction in IL4 and IL2 responses. In contrast, Jones et al. (51) reported increased IL5, IFN γ , IL-1 β , and TNF α production in patients who underwent peanut OIT. Th1 (IFN γ) responses have been noted to increase (51) or remain unchanged (46). Treg numbers (56, 57) and production of regulatory cytokines such as TGF β (58) were reported to increase with OIT, although one study noted only transient changes (45). More detailed characterization of OIT immune effects is required to better decipher the fundamental processes modulated by OIT.

Safety of OIT

Adverse effects are frequent with OIT but are generally mild, comprising localized (oral/perioral) or mild generalized allergic reactions involving the skin or gut (38). Severe allergic reactions with respiratory or cardiovascular compromise are less common. Reactions to OIT mostly occur during up dosing (38), but importantly may also occur to a previously tolerated dose (59, 60).

In a systematic review of cow's milk OIT trials (43), OIT was associated with increased risk of allergic reactions: lip and/or mouth pruritus (RR 34.4; 95% CI 4.8–244.7), perioral urticaria (RR 9.9; 95% CI 4.3–22.9), generalized erythema or urticaria (RR 12.1; 95% CI 3.0–49.1), abdominal pain, vomiting, diarrhea (RR 16.6; 95% CI 4.1–66.9), mild laryngospasm (RR 40.9; 95% CI 2.5–673.7), mild bronchospasm (RR 10.0; 95% CI 2.4–41.4), as well as an increased risk for requiring intramuscular epinephrine (RR 5.8; 95% CI 1.6–21.9) as compared to remaining on an elimination diet. Subjects on OIT were more likely to receive adrenaline injections as compared to those on an elimination diet (23 injections per 100 patients per year [range 5–40] vs. 0 injections per 100 patients per year) and more likely to require systemic corticosteroid (15 per 100 [range 4–62] vs. 1 per 100), but were less likely to have an exacerbation of eczema (8 per 100 [range 1–100] vs. 14 per 100).

Reactions to a previously tolerated dose of OIT during home dosing are common. Barbi et al. (59) presented their experience with OIT in 132 patients and reported reactions during home dosing in 63.7% (84/132), with 29.5% having 1–5 reactions, 23.5% having 5–15 reactions, and 10.7% reporting more than 15 reactions. Of concern, 47.7% of reactions during home dosing affected the respiratory tract, equating to a severe systemic reaction or anaphylaxis, while 28.8% of reactions involved the gastrointestinal tract and 23.5% were cutaneous. Common triggers for reactions to a previously tolerated dose include concurrent infection (particularly if febrile), physical activity within 2 h of a dose, taking a dose on an empty stomach, poorly controlled asthma, pollen season, and menses (59, 60). Of note, no trigger could be identified in 28.5% of

home reactions in the Barbi et al. series (59). Reactions may be minimized by withholding treatment if febrile, avoiding physical activity for 2 h after dosing, taking treatments after a meal, and maintaining optimal asthma control (60).

Future directions for OIT

Efforts to improve the safety and tolerogenic capacity of OIT are warranted to support the feasibility and effectiveness of OIT as a future treatment for food allergy in the clinical setting. Although OIT has been applied successfully to subjects with severe allergy (anaphylaxis) (39, 61), it may take many months to reach maintenance dose and subjects experience frequent reactions during the up dosing phase. The use of omalizumab, a recombinant humanized monoclonal antibody that binds to free circulating IgE, has been suggested as one approach to reduce allergic reactions during the rush and up dosing phases of OIT. Omalizumab binds to the CH ϵ 3 region of IgE and prevents binding of IgE to Fc ϵ RI and Fc ϵ RII receptors on the surface of mast cells and basophils thereby preventing mast cell and basophil activation and mediator release. A pilot study of omalizumab treatment together with cow's milk OIT showed improved tolerability of OIT (62). RCT evaluating the use of omalizumab with milk or peanut OIT are currently underway. Omalizumab may also modulate the allergic response to promote tolerance [reviewed in (63)].

Another approach that may improve safety of OIT is the use of extensively heated (baked) egg or milk in place of native allergen (64, 65). A large proportion of children allergic to egg or cow's milk are able to tolerate extensively heated forms of egg or milk while being reactive to raw egg or whole cow's milk. In such individuals, regular intake of baked egg or milk was associated with immune changes similar to those observed with standard OIT, including reduction in SPT wheal size, reduced allergen-specific IgE, and increased allergen-specific IgG4 (64–66), and treatment with baked egg was also suggested to promote tolerance to regular egg (67). However, these findings require confirmation in controlled studies. In the above reports, immune data were not available from control subjects so it is possible that some of these changes may reflect the natural history of egg or milk allergy. Similarly, Leonard et al. (67) examined the development of tolerance to regular egg in subjects with egg allergy who received baked-egg OIT if they were baked-egg tolerant (active group, $n = 79$) and included a comparison group of subjects with egg allergy ($n = 47$) who remained on a strict egg elimination diet; however, the outcome of tolerance was not assessed in a standardized way across both the active and comparison groups (67). In that study, egg-allergic subjects in the active group were challenged with baked egg, and those who were able to tolerate baked egg ($n = 56$ at study entry and $n = 70$ at end of study) were instructed to include 1–3 serves of baked egg each day in their diet, while those who were baked-egg reactive ($n = 23$ at study entry and $n = 9$ at end of study) remained on a strict elimination diet. Tolerance to baked or regular egg was then assessed by food challenge after ≥ 6 months if they were baked-egg tolerant and receiving baked egg or after

≥12 months if they were baked-egg reactive. The comparison group was followed in parallel with no specific plan for assessment of tolerance, and challenges were performed at variable times according to the recommendation of each patient's individual allergist. In an intention to treat analysis, egg-allergic subjects in the active group (comprising $n = 70$ who received baked-egg and $n = 9$ who remained on an elimination diet) were 4.7 (1.9–11.5) times more likely to develop tolerance to regular egg than those in the comparison group on an elimination diet. In the per protocol analysis, active group participants who were baked-egg tolerant and received baked egg ($n = 70$) were 18.3 (5.5–60.9) times more likely to develop tolerance to regular egg than the comparison group. These findings are impressive and suggest that baked-egg OIT may hasten the acquisition of tolerance to regular egg. Nevertheless, as stated above, the data must be interpreted with caution since the variable approach to assessment of tolerance across the active and comparison group participants introduces significant bias. RCT (ideally including a placebo group) to evaluate heated milk or egg OIT effects on development of tolerance and immune responses are warranted.

A new class of adjuvants, known as immune response modifiers, has emerged in the last decade and offers a strategy for enhancing tolerogenic responses associated with immunotherapy. Immune modifying adjuvants typically signal through pathogen recognition receptors and have been shown to promote T regulatory and Th1 responses. For example,

monophosphoryl lipid A, a bacterial cell wall component that signals through TLR4, and CPG-containing DNA allergen complexes were reported to enhance tolerogenic effects of pollen subcutaneous immunotherapy in humans (68–70). Various TLR ligands and probiotic bacteria used in conjunction with sub-lingual immunotherapy have also been demonstrated to enhance tolerogenic effects in mouse models of allergic airway disease (71–73). Similar effects might be expected in the setting of OIT. In this regard, we have recently completed a randomized placebo controlled trial of a probiotic in combination with OIT for the treatment of peanut allergy (ANZ Clinical Trials Registry #12608000594325), and results will be available in the near future.

Conclusion

Food allergies continue to increase exponentially and the search for therapies that can change the natural history of disease is a research priority. Food allergy occurs as a result of failure or loss of oral tolerance. Increased understanding of oral tolerance mechanisms may facilitate development of novel approaches to tolerance induction in food allergy. OIT offers a promising strategy for oral tolerance induction and treatment of food allergy. Clinical trials of OIT demonstrate effective desensitization, but the ability to induce long-lasting tolerance remains uncertain. Use of immune modifying adjuvants may pave the way to enhance the tolerogenic capacity of OIT, and trials are currently underway to explore this.

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