

# Oral tolerance, food allergy, and immunotherapy: Implications for future treatment

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The lumen of the gastrointestinal tract is exposed daily to an array of dietary proteins. The vast majority of proteins are tolerated through suppression of cellular or humoral responses, a process known as oral tolerance. However, in approximately 6% of children and 4% of adults in the United States, tolerance to a given dietary antigen either is not established or breaks down, resulting in food hypersensitivity. Although food allergies can result in sudden and life-threatening symptoms, their prevalence is remarkably low considering the complexities of the gut-associated mucosal system. Suppression involves signaling by an array of nonprofessional antigen-presenting cells, dendritic cells, and regulatory T cells, as well as lymphocyte anergy or deletion. Several factors, including antigen properties, route of exposure, and genetics and age of the host, contribute to the development of oral tolerance. Although the current standard of care for patients with food allergies is based on avoidance of the trigger, increased understanding of the mechanisms involved in tolerance has shifted focus of treatment and prevention toward inducing tolerance. Data from early-phase clinical trials suggest both sublingual and oral immunotherapy are effective in reducing sensitivity to allergens. In this article we review the mechanisms of tolerance, discuss aberrations in oral tolerance, and provide information on novel prevention and treatment paradigms for food allergy. (*J Allergy Clin Immunol* ■■■■;■■■:■■■-■■■.)

**Key words:** Immune tolerance, immunotherapy, food allergy, food hypersensitivity, T cells, TGF- $\beta$ , clonal anergy, forkhead box P3 protein, sublingual immunotherapy, oral immunotherapy

Food allergy affects 6% of children younger than 3 years of age and approximately 4% of adults in the United States.<sup>1</sup> Food-

## Abbreviations used

APC: Antigen-presenting cell  
FOXP3: Forkhead box P3  
IPEX: Immune dysregulation, polyendocrinopathy, enteropathy, X-linked  
OIT: Oral immunotherapy  
SLIT: Sublingual immunotherapy  
TLR: Toll-like receptor

induced anaphylaxis is the most common cause of anaphylaxis treated in hospital emergency departments.<sup>2</sup> The prevalence of certain food allergies, such as peanut, is increasing,<sup>3</sup> as is public awareness of the disease and the need for novel treatment strategies. At present, the standard of care for food allergy includes strict avoidance of food allergens and ready access to self-injectable epinephrine. The difficulty in avoiding food allergens and the potential for sudden and life-threatening reactions can diminish health-related quality of life for patients and their families.<sup>4-6</sup>

Although the effects of food allergies are substantial, their prevalence is remarkably low considering the complexities of the gut-associated mucosal system, where tolerance is the norm. The lumen of the gastrointestinal tract, which is the largest immunologic organ in the body,<sup>7</sup> is exposed daily to an array of bacteria and ingested proteins. Lining the gastrointestinal tract is a single layer of epithelium, and under that is a stroma of loose connective tissue populated by lymphocytes. A dietary protein antigen interacts with specific antigen-presenting cells (APCs), which help to activate regulatory T cells, usually resulting in a suppression of immune response. Thus oral tolerance is the specific suppression of cellular or humoral immune responses to an antigen by means of prior administration of the antigen through the oral route.<sup>7,8</sup> The response likely evolved as an analog of self-tolerance to prevent hypersensitivity reactions to food proteins and bacterial antigens present in the mucosal microbiota. Food hypersensitivity likely results from either a failure in establishing oral tolerance or a breakdown in existing tolerance.

For centuries, inducing tolerance has been used as a strategy for preventing allergic reactions. In 1829, Dakin<sup>9</sup> reported that certain populations of Native Americans ate poison ivy leaves to avoid contact hypersensitivity reaction to urushiol, and in a classic experiment, Wells<sup>10</sup> found that guinea pigs that were repeatedly fed protein from hen's eggs were protected from anaphylaxis when injected with the protein. More recently, understanding oral tolerance has been recognized as a key component in developing strategies for preventing and treating food allergies. In this article we review the mechanisms of oral tolerance, discuss aberrations in tolerance, and provide information on novel prevention and treatment paradigms for food allergy.

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## MECHANISMS OF TOLERANCE

Experiments in the mid-20th century established that oral feeding of an antigen can induce T cell–mediated inhibition of active immune responses.<sup>11</sup> Mice that are immunized and boosted subcutaneously with an antigen exhibit strong *in vitro* cell-mediated and antibody responses to the antigen. In contrast, mice that are first fed the antigen orally and then immunized subcutaneously have greatly reduced *in vitro* immune responses to the antigen. Transferring T cells from antigen-fed mice to naive mice also results in reduced *in vitro* immune responses to subcutaneous immunization.

Antigen exposure in the gut leads to protective local and systemic immunologic responses. The local noninflammatory secretory IgA antibody is the initial response that occurs in mucosal surfaces.<sup>12</sup> Subsequently, the systemic immune system can be primed, leading to production of serum antibodies and cell-mediated immunities that protect against the invading antigen on subsequent exposures. For most dietary proteins and commensal bacteria, a state of local and immunologic tolerance exists that prevents potentially damaging active immunity when the antigen is encountered on subsequent occasions. Food proteins present in the normal diet also play a critical role in stimulating maturation of the immune system. Mice that are reared on a balanced but protein-free diet have poorly developed gut-associated lymphoid tissue that resembles that of suckling mice.<sup>13</sup> Such mice also have low numbers of lymphocytes and levels of circulating IgG and IgA, as well as cytokine production consistent with T<sub>H</sub>2 responses.

At the interface of innate and adaptive immunity are Toll-like receptors (TLRs), which have a major role in downregulating or enhancing immune responses. Toll was first discovered as being involved in antifungal responses in *Drosophila* species,<sup>14</sup> and later the human homolog was found to induce production of inflammatory cytokines and expression of costimulatory molecules in human subjects.<sup>15</sup> After recognition of microbial pathogens, TLRs trigger induction of inflammatory cytokines, type I interferon, and chemokines.<sup>16</sup> TLR signaling also upregulates costimulatory molecules on specialized APCs called dendritic cells, a process that is essential for inducing pathogen-specific adaptive immune responses.<sup>17</sup>

Ingested dietary proteins are degraded and their conformational epitopes are destroyed by gastric acidity and luminal digestive enzymes, which often results in the destruction of immunogenic epitopes. In animal models, disrupting the process of digestion can disrupt tolerance and lead to hypersensitivity. Untreated BSA is immunogenic when administered to mice by means of ileal injection; however, administering a peptic digest of the protein in the same manner results in immune tolerance.<sup>18</sup> Ingestion of proteins that are protected from both acid and enzymatic digestion can interrupt already established tolerance. Barone et al<sup>19</sup> encapsulated the protein ovalbumin in water-soluble, low-pH acrylic microspheres to protect it from digestion. After feeding the encapsulated ovalbumin to mice previously tolerized to ovalbumin, total IgG anti-ovalbumin antibody and IgG1 titers were no higher than in water-fed control mice. Splenocyte proliferation was also increased in the mice who received encapsulated albumin. The mechanism behind this finding is not entirely clear; the microspheres may not only protect the protein from acid and enzymatic digestion, they may alter the site of protein entry into the digestive tract.

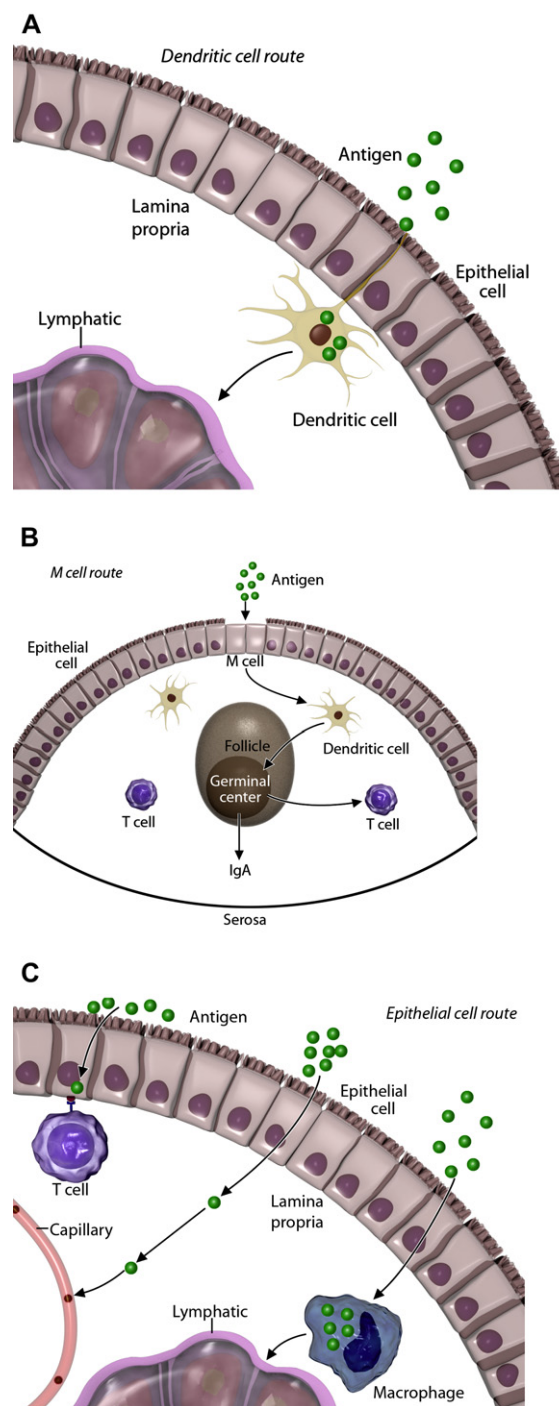


**FIG 1.** Antigen sampling in the gut. **A**, Dendritic cells extend processes through the epithelium and into the lumen. **B**, M cells overlying Peyer's patches take up particulate antigens and deliver them to subepithelial dendritic cells. **C**, Soluble antigens possibly cross the epithelium through transcellular or paracellular routes to encounter T cells or macrophages in the lamina propria. Modified with permission from Chehade M, Mayer L. Oral tolerance and its relation to food hypersensitivities. *J Allergy Clin Immunol* 2005;115:3-12.<sup>7</sup>

Proteins that are not digested and processed in the lumen of the gut will contact the epithelium and mucosal immune system beneath it in various manners (Fig 1). In the gut, dendritic cells can sample antigens by extending processes through the epithelium

and into the lumen. M cells that overlie Peyer's patches can take up particulate antigens and deliver them to subepithelial dendritic cells. Soluble antigens possibly cross the epithelium through transcellular or paracellular routes to encounter T cells or macrophages in the lamina propria. Dietary proteins that escape proteolysis in the gut can be taken up by intestinal epithelial cells. The epithelial cells can act as nonprofessional APCs given that they constitutively express MHC class 2 molecules on their basolateral membranes<sup>20,21</sup> and can present antigen to primed T cells.

There are 2 primary effector mechanisms for inducing oral tolerance: active suppression by regulatory T cells or clonal anergy or deletion (Fig 2). The primary factor that determines which will take place is the dose of antigen.<sup>22</sup> Low doses of antigen favor tolerance driven by regulatory cells; high doses favor anergy-driven tolerance. Regulatory T cells suppress immune responses through soluble or cell surface-associated downregulatory cytokines, such as IL-4, IL-10, and TGF- $\beta$ .<sup>7</sup> Suppression can be carried out by suppressor CD8<sup>+</sup> cells<sup>23</sup> or helper CD4<sup>+</sup> cells.<sup>24-26</sup> Antigen-specific regulatory cells migrate to lymphoid organs, where they inhibit the generation of effector cells, as well as to target organs, where they release non-antigen-specific cytokines.<sup>8</sup>

Defects in regulatory T-cell activity likely contribute to the development of food allergy. CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells mediate suppression through cell surface-bound TGF- $\beta$ ,<sup>27</sup> although suppressor function can possibly occur independently of TGF- $\beta$ .<sup>28</sup> CD4<sup>+</sup>CD25<sup>+</sup> cells express the transcription factor forkhead box P3 (FOXP3),<sup>29,30</sup> which is thought to help block T<sub>H</sub>1 and T<sub>H</sub>2 responses.<sup>31</sup> Mutations of the gene encoding FOXP3 result in a fatal disorder known as immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome.<sup>32</sup> A deletion in the noncoding region of FOXP3 that impairs mRNA splicing has been associated with a variant of IPEX syndrome that includes severe enteropathy, atopic dermatitis, and food allergies.<sup>33</sup>

In an analysis of children with milk-induced gastrointestinal diseases, milk-specific duodenal mucosal lymphocytes were cultured *in vitro* in the presence of milk. On restimulation with milk protein, the cells released the T<sub>H</sub>2-associated cytokines IL-5 and IL-13 but only very low amounts of TGF- $\beta$  and IL-10.<sup>34</sup>

Finally, among children with non-IgE-mediated allergy to cow's milk, which is frequently outgrown, there is evidence that the development of tolerance is associated with the appearance of circulating CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells. Children who outgrow their allergy have higher numbers of circulating CD4<sup>+</sup>CD25<sup>+</sup> T cells and decreased *in vitro* proliferative responses to bovine  $\beta$ -lactoglobulin in PBMCs than children who maintain clinically active allergy.<sup>35</sup> Depletion of CD25<sup>+</sup> cells from PBMCs of tolerant children leads to a 5-fold increase in *in vitro* proliferation against  $\beta$ -lactoglobulin.

High-dose tolerance is mediated by lymphocyte anergy or clonal deletion. Anergy can occur through T-cell receptor ligation in the absence of costimulatory signals provided by soluble cytokines, such as IL-2, or by interactions between receptors on T cells (CD28) and counterreceptors on APCs (CD80 and CD86).<sup>36</sup> Clonal deletion occurs by means of FAS-mediated apoptosis, which can be blocked by the proinflammatory cytokine IL-12.<sup>38</sup>

It has been suggested that low- and high-dose tolerance might not be mutually exclusive and might have overlapping functionality.<sup>8</sup> Apoptotic T cells release TGF- $\beta$  in both latent and bioactive forms, and macrophages produce TGF- $\beta$  on ingesting

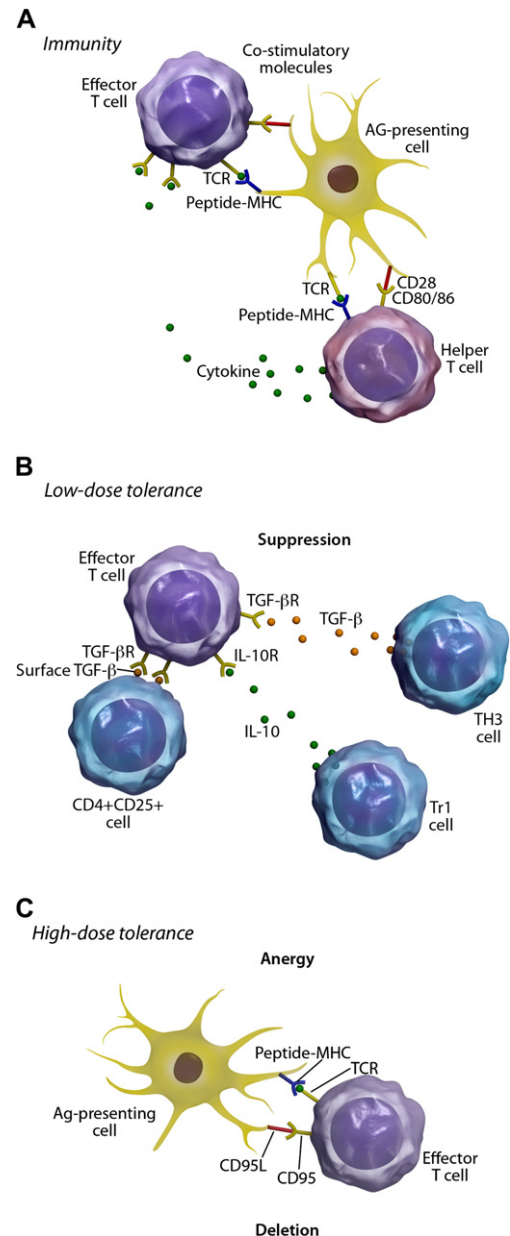


**FIG 2.** Mechanisms of oral tolerance. **A**, Immune responses require T-cell receptor ligation with peptide-MHC complexes in the presence of appropriate costimulatory molecules (CD80 and CD86) and cytokines. **B**, Low doses of antigen favor tolerance driven by regulatory cells, which suppress immune responses through soluble or cell surface-associated downregulatory cytokines, such as IL-4, IL-10, and TGF- $\beta$ . **C**, High-dose tolerance is mediated by lymphocyte anergy or clonal deletion. Anergy can occur through T-cell receptor ligation in the absence of costimulatory signals. Clonal deletion occurs by means of FAS-mediated apoptosis (CD95). TCR, T-cell receptor; Ag, antigen. Modified with permission from Chehade M, Mayer L. Oral tolerance and its relation to food hypersensitivities. *J Allergy Clin Immunol* 2005;115:3-12.<sup>7</sup>

apoptotic cells.<sup>39,40</sup> Secretion of TGF- $\beta$  can be induced by treating T cells with anticytotoxic T-lymphocyte antigen 4 antibodies, despite the evidence that anticytotoxic T-lymphocyte antigen 4 also appears to be involved in the induction of clonal anergy *in vivo*.<sup>41,42</sup> The secretion of TGF- $\beta$  through the various mechanisms of clonal anergy and deletion can contribute to an immunosuppressive environment in the gut.

## FACTORS IMPORTANT IN TOLERANCE

Several factors, including antigen properties, route of exposure, and genetics and age of the host, contribute to the development of oral tolerance. In general, soluble antigen is more tolerogenic than particulate antigen, yet paradoxically, most food allergens are soluble proteins. Solubility can change during food preparation, and peanut allergens become less soluble with progressive roasting, a process that increases the capacity of peanut-specific IgE binding to the protein.<sup>43</sup> The route of antigen exposure is also important because food antigen exposure by means other than ingestion can cause hypersensitivity. There is some evidence in human subjects that using skin lotions containing peanut oils can lead to the development of peanut allergy,<sup>44</sup> although these data have not been confirmed as yet. In mice, epicutaneous or epidermal exposure to peanut protein prevents oral tolerance and enhances allergic sensitization.<sup>45</sup> Epicutaneous exposure to peanut can induce potent T<sub>H</sub>2-type immunity with high levels of IL-4 and serum IgE in naive mice and partial sensitization in mice with existing tolerance.

In murine models of oral tolerance induction, genetics can contribute to the development of food hypersensitivity. In a study of allergen gene immunization for preventing food allergy, 3 strains of mice, C3H/HeSn, AKR/J, and BALB/c, received injections of plasmid DNA encoding Ara h 2, one of the major peanut allergens.<sup>46</sup> Subsequent injection of peanut protein 3 or 5 weeks later resulted in anaphylaxis in all of the C3H/HeSn mice (n = 15) but none of the AKR/J (n = 18) or BALB/c (n = 16) mice. In contrast to the C3H/HeSn mice, the AKR/J and BALB/c mice had increased concentrations of IgG2a but not IgG1 or IgE. Morafo et al<sup>47</sup> sensitized C3H/HeJ and BALB/c mice to cow's milk or peanut protein by means of intragastric administration. On food challenge, 87% of C3H/HeJ mice experienced anaphylaxis from cow's milk, and 100% experienced anaphylaxis from peanuts. In contrast, BALB/c mice did not experience hypersensitivity to either cow's milk or peanuts. Splenocytes from C3H/HeJ mice sensitized to cow's milk or peanut exhibited significantly increased IL-4 and IL-10 secretion, yet splenocytes from BALB/c mice exhibited significantly increased IFN- $\gamma$  levels. Susceptibility to food allergy might be related to differential responses in various strains of mice, with a T<sub>H</sub>2-dominant response linked to food allergy and a T<sub>H</sub>1-dominant response linked to tolerance.

Age of exposure is also important in the development of tolerance. In mice, feeding weight-related doses of ovalbumin to either neonates or adults for the first time results in dramatically different outcomes. Feeding a weight-related dose of ovalbumin to mice within the first week of life results in priming for both humoral and cell-mediated immune responses, although adult animals treated the same way experience tolerance.<sup>48</sup> The progression from sensitivity to tolerance appears to shift gradually with age, with tolerance becoming the norm as mice become 2 to 3 weeks old, the time when they are normally weaned.

Recent research has investigated whether certain allergens possess innate immunostimulatory properties. The mammalian immune system has evolved mechanisms to recognize bacterial proteins in association with pathogen-associated molecular patterns that induce either T<sub>H</sub>1 or T<sub>H</sub>2 responses. It has been hypothesized that other motifs on nonmammalian proteins could similarly be recognized as nonself and induce T<sub>H</sub>2 responses in individuals susceptible to allergies. Complex mannose glycans

are lacking from mammalian glycoproteins but are commonly found on those of plants, arthropods, and helminths. Deglycosylated peanut antigen, in contrast to untreated peanut antigen, does not activate monocyte-derived dendritic cells as measured by MHC/costimulatory molecule upregulation and by ability to drive T-cell proliferation.<sup>49</sup>

## PARADIGMS FOR INDUCING TOLERANCE

Caseins, which account for about 80% of protein in cow's milk, are the major allergens responsible for cow's milk allergy. Cow's milk allergy is a significant problem in young children but is often outgrown within the first 3 to 4 years of life. To better understand the natural history of cow's milk allergy, Chatchatee et al<sup>50</sup> identified IgE- and IgG-binding epitopes on 2 different caseins:  $\beta$ -casein and  $\kappa$ -casein. They isolated 9 IgE- and IgG-binding epitopes each on  $\beta$ -casein and 8 IgE- and 4 IgG-binding epitopes on  $\kappa$ -casein. Three of the IgE binding regions on  $\beta$ -casein and 6 on  $\kappa$ -casein were recognized by the majority of patients among older but not younger patients, suggesting that patients who do not outgrow cow's milk allergy experience "epitope spreading," or increased antigen recognition. A follow-up study confirmed that differences in epitope recognition appear to be useful in predicting patients whose allergies will persist.<sup>51</sup>

Patients whose allergies are likely to persist, as well as those who are susceptible to food allergies, are potential candidates for allergen immunotherapy. Injection of food allergens has been explored as a mechanism for immunotherapy, but the technique has been found to be unsafe.<sup>52</sup> Several alternatives to antigen injection, including injection with engineered antigen or ingestion of antigen through the gastrointestinal route, are currently being evaluated (Table I).

Immunization with engineered peanut protein allergens that have altered IgE-epitope binding sites is a strategy that has showed positive results in mice. Through recombinant genetic techniques, Li et al<sup>53</sup> altered single amino acids within epitope-binding regions of Ara h 1, Ara h 2, and Ara h 3 proteins. A single dose of the recombinant proteins was administered rectally to C3H/HeJ mice. During a food challenge with peanut 10 weeks later, mice that received the recombinant proteins were protected from anaphylactic symptoms. In mice that received the highest dose of protein, IL-4, IL-13, IL-5, and IL-10 production was significantly decreased, and IFN- $\gamma$  and TGF- $\beta$  production was significantly increased.

Establishment of tolerance in infants and small children might be important for preventing the development of food allergies. Interestingly, children in countries that have peanut snacks that are safe for infants to consume have relatively low rates of peanut allergies.<sup>54</sup> Randomized controlled trials are underway to test whether high doses of peanut protein in high-risk infants are more effective at preventing peanut allergy than avoidance therapy. The Learning Early About Peanut Allergy Study is enrolling 480 children aged 4 to 10 months who have eczema and are allergic to egg (such children have a 20% chance of having peanut allergy). Each child will be randomly assigned to either avoid consumption of peanuts or to be fed an age-appropriate peanut snack 3 times per week (about 6 g of peanut per week).<sup>55</sup> The rates of development of peanut allergy by age 5 years will be compared.

Sublingual immunotherapy (SLIT) and oral immunotherapy (OIT) are strategies that have been tested for their ability to induce desensitization in patients with food hypersensitivities

**TABLE I.** Strategies for inducing oral tolerance

| Therapy                                               | Research stage | Route      | Immunologic mechanism                                     |
|-------------------------------------------------------|----------------|------------|-----------------------------------------------------------|
| Mutated protein immunotherapy                         | Preclinical    | Rectal     | Possibly switch from T <sub>H</sub> 2 to T <sub>H</sub> 1 |
| High dose of allergen in at-risk infants (LEAP Study) | Clinical       | Oral       | Early induction of T <sub>H</sub> 1 responses             |
| SLIT                                                  | Clinical       | Sublingual | Possibly switch from T <sub>H</sub> 2 to T <sub>H</sub> 1 |
| OIT                                                   | Clinical       | Oral       | Possibly switch from T <sub>H</sub> 2 to T <sub>H</sub> 1 |

LEAP, Learning Early About Peanut Allergy.

**TABLE II.** Selected SLIT and OIT studies

|                                                  | Allergen received<br>(no. of subjects) | Length of therapy | Efficacy                                                                                                                                        |
|--------------------------------------------------|----------------------------------------|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| Sublingual<br>Enrique et al (2005) <sup>56</sup> | Hazelnut (11)                          | 8-12 wk           | Five (45%) of 11 reached highest dose (20 g) in food challenge.                                                                                 |
| Oral<br>Patriarca et al (2003) <sup>57</sup>     | Cow's milk (24)                        | 18 mo             | Desensitization was successful in 45 (77%) of 58 treatments.                                                                                    |
|                                                  | Whole egg (13)                         |                   |                                                                                                                                                 |
|                                                  | Albumin (3)                            |                   |                                                                                                                                                 |
|                                                  | Fish (10)                              |                   |                                                                                                                                                 |
|                                                  | Orange (2)                             |                   |                                                                                                                                                 |
|                                                  | Peanut (1)                             |                   |                                                                                                                                                 |
|                                                  | Corn (1)                               |                   |                                                                                                                                                 |
|                                                  | Peach (1)                              |                   |                                                                                                                                                 |
|                                                  | Apple (1)                              |                   |                                                                                                                                                 |
|                                                  | Lettuce (1)                            |                   |                                                                                                                                                 |
|                                                  | Beans (1)                              |                   |                                                                                                                                                 |
| Meglio et al (2004) <sup>58</sup>                | Cow's milk (21)                        | 6 mo              | Fifteen (71%) of 21 achieved daily intake of 200 mL; 3 tolerated 40-80 mL/d.                                                                    |
| Buchanan et al (2007) <sup>59</sup>              | Egg (7)                                | 24 mo             | Four (57%) of 7 passed food challenge with 8 g of egg at conclusion of therapy; 2 passed second challenge 3-4 mo later.                         |
| Staden et al (2007) <sup>60</sup>                | Cow's milk or egg (45)                 | 11-59 mo          | Nine (36%) of 25 who received allergen achieved permanent tolerance; 7 (35%) of 20 who underwent elimination diet achieved permanent tolerance. |
| Longo et al (2008) <sup>61</sup>                 | Cow's milk (30)                        | 12 mo             | Eleven (36%) of 30 achieved daily intake of 150 mL/d; 16 (54%) of 30 tolerated 5-150 mL/d.                                                      |
| Nash et al (ongoing)                             | Peanut (24)                            | 18 mo             | To date, 18 (90%) of 20 passed food challenge to 3.9 g of peanut protein.                                                                       |

(Table II).<sup>56-61</sup> Both methods generally involve administering small (usually micrograms, milligrams, grams) yet increasing doses of antigen in a controlled setting followed by regular home dosing of a maximum tolerated amount of antigen. Treatment is followed by an open or blinded food challenge with antigen or placebo. In a study of SLIT for hazelnut allergy, 23 patients received either hazelnut extract or placebo for 8 to 12 weeks.<sup>56</sup> In the subsequent food challenge, the mean hazelnut quantity that provoked symptoms increased 9 g from baseline in the hazelnut group versus 0.6 g in the placebo group. Subjects in the hazelnut group also experienced increases in IgG4 and IL-10 levels, although none in the placebo group experienced these increases.

In a standardized OIT protocol for treatment of various food allergies, desensitization occurred in 77% of treatments.<sup>57</sup> The most common food allergy among subjects was milk, followed by egg and fish. In comparison with age-matched control subjects with food allergy, subjects receiving OIT experienced a significant decrease in food-specific IgE levels and an increase in specific IgG4 levels. Meglio et al<sup>58</sup> used an OIT protocol in children with proved IgE-mediated sensitivity to milk. In 6 months, 15 of 21 children were fully desensitized; 3 children were partially desensitized. Even partial desensitization dramatically reduced the risk of severe reactions after accidental or unnoticed ingestion of cow's milk at low quantities. More recently, 7

subjects with egg allergy completed a 24-month protocol for egg OIT.<sup>59</sup> Four of the 7 subjects passed a double-blind, placebo-controlled food challenge to 10 g of egg at the conclusion of the therapy. The remaining 3 subjects had significantly increased tolerance to egg.

Although both SLIT and OIT have shown positive results, most of the studies reported to date have lacked control groups, and it has been suggested that success of the therapy might be due to spontaneous desensitization that can occur with age. In 2005, Rolinck-Werninghaus et al<sup>62</sup> reported initial results for 3 patients enrolled in a randomized, placebo-controlled study of oral tolerance induction therapy for cow's milk or egg allergy. Home daily dosing was conducted until patients achieved tolerance to 250 mL of milk or 4.5 g of egg protein, which happened after 37 to 52 weeks. Daily dosing was followed by an elimination diet for 2 months and then a double-blind, placebo-controlled food challenge. Of the 2 patients who underwent the final food challenge, both experienced moderate systemic allergic reactions similar to their symptoms before therapy.

Results from the larger study of 45 patients, which followed the same protocol, have recently been published.<sup>60</sup> At the follow-up food challenge, 9 (36%) of 25 children showed permanent tolerance in the tolerance induction group versus 7 (35%) of 20 in the elimination diet control group. The results suggest that oral tolerance induction therapy might not alter the natural course of

tolerance development. However, in the tolerance induction group, 7 children were considered either partial responders or responders when receiving regular antigen intake, boosting the rate of any response to 64%. Allergen-specific IgE levels decreased significantly in children who had natural tolerance during the elimination diet and those who received induction therapy. Even though partially tolerant patients would need a regular intake of allergen to maintain desensitization, this could protect patients against severe reactions or those resulting from accidental exposure to antigen. Cow's milk and egg allergies are commonly outgrown, which might explain the similar values in the treatment induction and control groups.

Indeed, a controlled study of oral tolerance therapy for children with severe allergy to cow's milk found significant differences between those who underwent therapy and those who followed an elimination diet.<sup>61</sup> Sixty children aged 5 years or older with a history of severe allergic reactions and very high levels of IgE specific to cow's milk protein were divided into 2 groups. One group underwent specific oral tolerance induction to cow's milk; the other was kept on a milk-free diet. After 1 year, 36% of children in the treatment group were completely tolerant to milk (at least 150 mL daily), 54% could tolerate 5 to 150 mL of milk daily, and 10% did not complete the protocol because of persistent respiratory or abdominal complaints. Tolerance was confirmed in an open feeding observed by investigators. In contrast, in the group that maintained an elimination diet, all 30 patients failed a double-blind, placebo-controlled food challenge after 1 year. Half of the patients in the treatment group, had significant decreases in IgE levels specific to cow's milk at 6 months and 12 months. In the control group, milk-specific IgE levels remained essentially unchanged, and none of the children spontaneously acquired tolerance.

In an ongoing study of children with peanut allergy, which is rarely outgrown, we are seeing similarly positive results with peanut OIT. Twenty-four children have been enrolled in a 24-month protocol that ends with an open food challenge to 3.9 g of peanut protein. To date, 20 subjects have completed the study, and 18 (90%) of them ingested 3.9 g of peanut protein during the challenge. During the study, subjects experienced typical allergic symptoms, primarily in the initial phases of therapy. Peanut-specific IgE levels increased initially but then decreased at 12 and 18 months, whereas peanut-specific IgG4 levels increased significantly throughout the study. The initial results suggest that peanut OIT can safely establish desensitization in children with peanut allergy, but it remains to be seen whether clinical tolerance will develop after this treatment.

To date, therapy aimed at inducing tolerance has been safe and well tolerated, with allergic reactions controlled by antihistamines, steroids, or epinephrine. However, treatment protocols have been initiated in highly supervised research settings and with small numbers of patients. Because the approach might place patients at risk for severe reactions, induction of tolerance should not be tried in clinical practice settings at this time.

## CONCLUSIONS

Understanding tolerance versus hypersensitivity is critical for the treatment of patients who have food allergies and for those who are susceptible to the disease. Further studies are needed to better understand the effectiveness of inducing tolerance to various allergens, the optimal mode of delivering antigen, and

whether induction can result in long-term tolerance versus short-term desensitization. SLIT and OIT strategies appear to be effective in inducing short-term desensitization, although they are not ready for implementation in the clinical setting, and randomized, placebo-controlled studies are needed to determine whether these strategies induce long-term tolerance. Such studies should include evaluation of cellular, humoral, and basophil/mast cell responses over time. As the molecular mechanisms of tolerance are further elucidated, the future for new therapeutic approaches for food allergy is promising.

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