

# Food Allergy Therapy: Is a Cure Within Reach?

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## KEYWORDS

- Food allergy • Immunotherapy • Oral immunotherapy
- Oral desensitization • Milk allergy • Peanut allergy
- Egg allergy • Food allergy therapy

Food allergy is a growing public health problem.<sup>1</sup> In the United States, it is estimated that 3.9% of general population under 18 years of age is affected by food allergies; the prevalence increased by 18% from 1997 to 2007.<sup>2</sup> Currently, the only treatment of food allergy relies on strict food avoidance, dietary management to avoid nutritional deficiencies, and prompt emergency treatment of acute reactions. There is an unmet medical need for an effective food allergy therapy; thus, development of therapeutic interventions for food allergy is a top research priority. Studies concentrate on the foods most commonly implicated in severe IgE-mediated anaphylactic reactions (peanut, tree nuts, and shellfish) and the most common food allergens, such as cow's milk and hen's egg.<sup>3</sup> The promising therapies under investigation can be classified as food allergen-nonspecific and food allergen-specific.<sup>4</sup> The food allergen-nonspecific therapies for food-induced anaphylaxis include monoclonal anti-IgE antibodies, which increase the threshold dose for peanut in peanut-allergic individuals, and Chinese herbs, which prevent peanut anaphylaxis in an animal model and are currently being evaluated in human studies (**Table 1**). The food allergen-specific therapies include oral immunotherapy (OIT), sublingual immunotherapy (SLIT), and epicutaneous immunotherapy (EPIT) with native food allergens (**Table 2**) and mutated recombinant proteins, which have decreased IgE-binding activity, coadministered within heat-killed *Escherichia coli* to generate maximum immune response (**Table 3**).

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The authors have nothing to disclose.

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Pediatr Clin N Am 58 (2011) 511–530

doi:[10.1016/j.pcl.2011.02.010](https://doi.org/10.1016/j.pcl.2011.02.010)

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| Table 1<br>Allergen-nonspecific therapy for food allergy |                                                                                                                                                                                                                 |                                                                                                                                    |                                                                                                                                                                                                                      |
|----------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Therapy                                                  | Mechanism of Action                                                                                                                                                                                             | Effects                                                                                                                            | Comments                                                                                                                                                                                                             |
| Monoclonal anti-IgE                                      | Binds to circulating IgE, prevents IgE deposition on mast cells, and blocks degranulation. Interferes with the facilitated antigen presentation by B-cell and dendritic cells.                                  | Improves symptoms of asthma and allergic rhinitis; provides protection against peanut anaphylaxis in 75% of treated patients.      | Subcutaneous at monthly or 2-week intervals, unknown long-term consequences of IgE elimination; food nonspecific; ongoing studies of combined anti-IgE and milk OIT in children                                      |
| Traditional TCM                                          | Down-regulation of T <sub>H</sub> 2 cytokines (IL-4, IL-5, and IL-13), up-regulation of T <sub>H</sub> 1 cytokines (IFN- $\gamma$ and IL-12), decreased allergen IgE, decreased T-cell proliferation to peanut. | Reverses allergic inflammation in the airways, affords prolonged protection from peanut anaphylaxis (for ~half of mouse lifespan). | Oral, generally safe and well tolerated; current studies focus on identification of the crucial active herbal components in the multiherb formulas and establishing optimal dosing in phase I and II clinical trials |

WHO NEEDS FOOD ALLERGY THERAPY?

Subjects at high risk for severe anaphylaxis and those unlikely to outgrow food allergy spontaneously are most in need of food allergy therapy. Traditional allergy tests (measurement of food allergen-specific IgE antibodies in serum or skin prick test) do not reliably predict the severity of future reactions or the spontaneous development of tolerance. The severity of food-allergic reactions may relate to the diversity of the immune response to IgE-binding epitopes on food allergens (Fig. 1). In a peptide microarray-based immunoassay, children with severe reactions to peanut and milk had IgE antibodies that bound to higher number of epitopes than children with mild reactions.<sup>5–7</sup> Persistent milk-allergic children had increased epitope diversity compared with those who outgrew their allergy.<sup>7</sup> Using a competitive peptide microarray assay, allergic patients demonstrated a combination of high-affinity and low-affinity IgE binding whereas those who had outgrown their milk allergy had primarily low-affinity binding.<sup>7</sup> Similarly, persistent egg allergy was associated with recognition of the sequential epitopes on ovomucoid, the major egg white allergen. Subjects who generated IgE antibody responses against both the conformational and sequential epitopes of ovomucoid were likely to have persistent egg allergy. In contrast, subjects who generated IgE antibody responses predominantly against the conformational epitopes of ovomucoid were more likely to have transient egg allergy.<sup>8</sup> Recognition of the specific casein epitopes might identify children at risk for more persistent milk allergy.<sup>9</sup> Persistence of food allergy might be related to high lifetime peak values of food-specific serum IgE antibodies. Two reports describing the natural history of milk and egg allergy in children with multiple food allergies observed that few children with peak milk-specific or egg white-specific IgE antibody levels greater than or equal to 50 kilounits of antibody (kU<sub>A</sub>)/L (UniCAP, Phadia, Uppsala, Sweden) outgrew their respective allergy by teenage years.<sup>10,11</sup>

Table 2

## Native allergen immunotherapy for food allergy

| Therapy                                              | Mechanism of Action                                                                                                                                                     | Effects                                                                                                                                                                 | Comments                                                                                                                                                                                                                              |
|------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Subcutaneous</b>                                  |                                                                                                                                                                         |                                                                                                                                                                         |                                                                                                                                                                                                                                       |
| Conventional peanut immunotherapy                    | Altered T-cell responses, up-regulation of suppressor cells                                                                                                             | Increased oral peanut tolerance                                                                                                                                         | Subcutaneous injections of gradually increasing doses of allergen; unacceptably high rate of serious adverse events                                                                                                                   |
| Birch pollen immunotherapy for oral allergy to apple | Marked reduction in skin test reactivity to raw apple; effect of immunotherapy inversely correlated with baseline skin reactivity but not with serum apple or birch IgE | Significant reduction or total resolution of oral allergy symptoms to raw Golden Delicious apple in a subset of patients receiving immunotherapy for at least 12 months | Clinical effect lasting for up to 30 months after discontinuation in >50% of patients                                                                                                                                                 |
| <b>Oral/Sublingual</b>                               |                                                                                                                                                                         |                                                                                                                                                                         |                                                                                                                                                                                                                                       |
| OIT                                                  | Decreased: skin test reactivity, food IgE, and IL-4 production by food-specific PBMCs<br>Increased: food IgG, IgA, CD4 <sup>+</sup> CD23 high T cells; IL-10            | Oral food desensitization or increased threshold dose of food for clinical reactions up to 6 months; short-term success rate approximately 75%                          | No long-term follow-up data; many patients experience recurrence of symptoms if food not ingested on a daily basis. Significant rate of moderate to severe adverse reactions; convenience of home administration of maintenance doses |
| SLIT                                                 | Serum hazelnut IgG4 and total IL-10 increased in treated group; no change in hazelnut IgE                                                                               | Oral food desensitization or increased threshold dose on oral hazelnut challenge                                                                                        | Systemic side effects rate 0.2% during rush buildup phase; adverse reaction rate less than with OIT; no long-term follow-up                                                                                                           |

*Abbreviation:* PBMCs; peripheral blood mononuclear cells.

**Table 3**  
**Engineered allergen immunotherapy for food allergy**

|                                                                        | Mechanism of Action                                                                                                                                        | Effects                                                                                                                                                                  | Comments                                                                                                                                                                                                                                                                                          |
|------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Approaches Actively Investigated</b>                                |                                                                                                                                                            |                                                                                                                                                                          |                                                                                                                                                                                                                                                                                                   |
| Engineered recombinant peanut IT                                       | Binding to mast cells eliminated or markedly decreased, T-cell responses comparable to native peanut allergens                                             | Protection against peanut anaphylaxis in mice                                                                                                                            | Improved safety profile compared with conventional IT, requires identification of IgE-binding sites                                                                                                                                                                                               |
| Heat-killed bacteria mixed with or expressing modified peanut proteins | Upregulation of T <sub>H</sub> 1 and T-regulatory cytokine responses                                                                                       | Protection against peanut anaphylaxis in mice, lasting up to 10 weeks after treatment                                                                                    | Concern for toxicity of bacterial adjuvants, excessive T <sub>H</sub> 1 stimulation, and potential for autoimmunity; heat-killed <i>E coli</i> expressing modified peanut allergens administered rectally viewed as the safest approach for future human studies. Human studies in adults ongoing |
| <b>Approaches No Longer Actively Investigated<sup>a</sup></b>          |                                                                                                                                                            |                                                                                                                                                                          |                                                                                                                                                                                                                                                                                                   |
| Peptide immunotherapy <sup>68</sup>                                    | Overlapping peptides (10–20 amino acids long) that represent the entire sequence of allergen. Binding to mast cells eliminated, T-cell responses preserved | Protection against peanut anaphylaxis in mice                                                                                                                            | Improved safety profile compared with conventional IT, does not require identification of IgE-binding epitopes; not feasible because the Food and Drug Administration requires quantification of each peptide within the peptide mixture; too expensive.                                          |
| Plasmid DNA-based immunotherapy <sup>69–71</sup>                       | Induces prolonged humoral and cellular responses due to CpG motifs in the DNA backbone                                                                     | Protection against peanut anaphylaxis in sensitized AKR/J mice but induction of anaphylaxis in C3H/HeJ (H-2 <sup>K</sup> ) mice; no effect on peanut-IgE antibody levels | Serious concerns regarding safety in view of strain-dependent effects in mice, concern for excessive T <sub>H</sub> 1 stimulation and autoimmunity                                                                                                                                                |
| Immunostimulatory sequences (ISS-ODN) <sup>72</sup>                    | Potent stimulation of T <sub>H</sub> 1 via activation of antigen-presenting cells, natural killer cells, and B cells; increased T <sub>H</sub> 1 cytokines | Protection against peanut sensitization in mice                                                                                                                          | Not shown to reverse established peanut allergy, concern for excessive T <sub>H</sub> 1 stimulation, and potential for autoimmunity                                                                                                                                                               |

**Abbreviations:** ISS-ODN, immunostimulatory sequences-oligodeoxynucleotides; IT, immunotherapy.

<sup>a</sup> These three additional immunomodulatory approaches to peanut allergy were explored in the animal studies but subsequently were abandoned in favor of other treatments due to practical considerations. It is possible that the peptide immunotherapeutic approach will be revisited when the most relevant epitopes for T cells on major peanut allergens are identified and the vaccine contains only the selected peptides that represent T-cell epitopes.

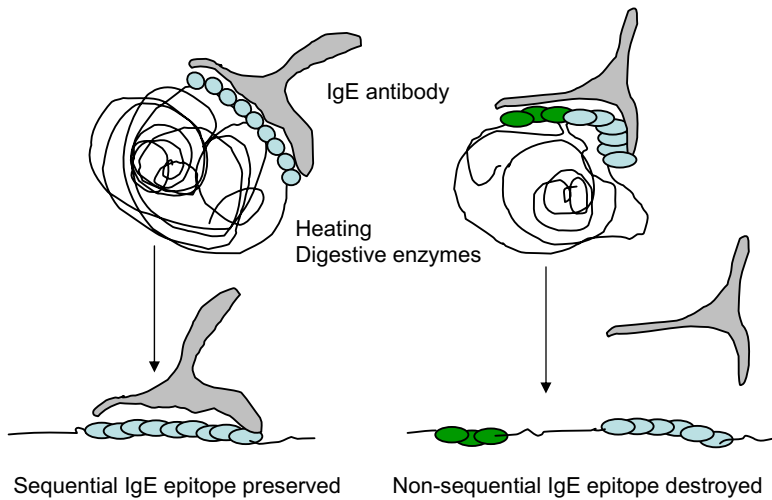


**Fig. 1.** Allergenic IgE antibody-binding epitopes. Children who generate IgE antibodies predominantly directed at conformational epitopes are more likely to have transient egg allergy. Children who generate IgE antibodies also against sequential epitopes are more likely to have persistent milk and egg allergy. Children who generate IgE antibodies against a large number of sequential epitopes are more likely to have more severe reactions.

## FOOD ALLERGEN-NONSPECIFIC THERAPY

### *Humanized Monoclonal Anti-IgE*

Humanized monoclonal anti-IgE antibodies bind to the constant region of IgE antibody molecules and prevent the IgE molecules from binding to high-affinity receptors,  $Fc\epsilon R1s$ , expressed on the surface of mast cells and basophils, and low-affinity receptors,  $Fc\epsilon R2s$ , expressed on B cells, dendritic cells, and intestinal epithelial cells. Anti-IgE cannot interact with IgE molecules when they are bound to IgE receptors and, therefore, cannot induce mast cell or basophil degranulation by cross-linking IgE; thus, the risk of immediate allergic reactions is reduced.<sup>12</sup> A multicenter randomized trial evaluated humanized monoclonal anti-IgE mouse IgG1 antibody (TNX-901) in 84 patients with a history of peanut allergy.<sup>13</sup> Baseline threshold dose of peanut protein necessary to elicit objective symptoms was established. Subjects were randomized to receive four doses of either humanized monoclonal antibody TNX-901 (150, 300, or 450 mg) or placebo subcutaneously every 4 weeks. They underwent a second oral peanut challenge within 2 to 4 weeks after the fourth dose. The mean amount of peanut flour that elicited objective symptoms and resulted in stopping of the food challenge (the sensitivity threshold) increased in all groups, with an apparent dose response, but was statistically significant only in the highest anti-IgE dose (450 mg) group. In this group, the sensitivity threshold increased from approximately one-half of a peanut kernel (178 mg) to almost 9 peanut kernels (2805 mg). Approximately 25% of subjects treated even with the highest dose of TNX-901, however, showed no change in their sensitivity threshold. A clinical trial of a different anti-IgE humanized IgG1 antibody (omalizumab) in peanut-allergic children older than 6 years of age was discontinued prematurely due to safety concerns related to severe anaphylactic reactions that occurred during the initial peanut challenge.

Combined treatment with anti-IgE and specific food allergen OIT may be a safer option because of the potential ability of anti-IgE to decrease immediate side effects

of allergen OIT.<sup>14</sup> Studies of a combination therapy are ongoing in children and adults with milk allergy.

### ***Traditional Chinese Medicine***

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Herbal remedies have been successfully used in China for centuries, although not for food allergies. The mechanism of action of traditional Chinese medicine (TCM) is unknown and TCM has not been systematically evaluated in randomized clinical trials. Food allergy herbal formula (FAHF)-1, a mixture of 11 herbs that has been used by TCM for treatment of parasitic infections, gastroenteritis, and asthma, was tested in a mouse model of peanut allergy.<sup>15</sup> Mouse models of food allergy were essential in the early stages of development of novel therapies for food allergy because human studies were considered unsafe due to the risk for anaphylaxis. Mouse models of peanut allergy mimicked human peanut allergy by the oral route of sensitization, symptoms of anaphylaxis after ingestion, generation of peanut-specific IgE antibodies, and release of allergic mediators (histamine) during the peanut challenge. FAHF-1 protected peanut-allergic mice against peanut-induced anaphylaxis and reduced mast cell degranulation and histamine release. Peanut-specific serum IgE levels decreased significantly after 2 weeks of treatment and remained lower 4 weeks after discontinuation of treatment. FAHF-1 had no toxic effects on liver or kidneys even at the doses that significantly exceeded the therapeutic dose.

A modified formula, FAHF-2, composed of 9 herbs, completely blocked anaphylaxis to peanut challenge up to 5 months after therapy.<sup>16</sup> A phase I clinical safety trial in adults 12 to 45 years of age with peanut and tree nut allergy was recently completed. FAHF-2 was found to be safe and well tolerated.<sup>17</sup> A phase II extended safety and efficacy trial is currently enrolling subjects 12 to 45 years of age with peanut, tree nut, sesame, fish, or shellfish allergy. FAHF-2 is expected to exert a similar protective effect against a variety of unrelated foods. The individual active substances in each herb are being identified, their mechanism of action characterized, and potency standardized. Each individual herb exerts some protective effect, but none offers equivalent protection from anaphylaxis compared with the complete FAHF-2 mixture of 9 herbs. The therapeutic effect of FAHF-2 seems to be in large part mediated by CD8<sup>+</sup> T cells producing interferon (IFN)- $\gamma$ .<sup>18,19</sup>

### **ALLERGEN-SPECIFIC IMMUNOTHERAPY**

Immunotherapy involves administration of allergens with or without adjuvants that skew the immune responses away from T<sub>H</sub>2 proallergic responses. In traditional environmental allergen immunotherapy, the dose escalation (also referred to as buildup phase) may be rushed over 1 to a few days (typically done in the hospital) or may last 4 to 6 months (typically done in the office). The maintenance phase begins when the highest tolerated dose has been reached. Maintenance dose is continued for extended periods of time; in subcutaneous allergen immunotherapy, maintenance dose is administered in the office; in OIT and SLIT, maintenance dose is administered at home.

### ***Desensitization Versus Permanent Oral Tolerance***

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Desensitization is distinct from permanent oral tolerance. In a desensitized state, the protective effect depends on daily, uninterrupted exposure to the allergen; however, when the dosing is interrupted or discontinued, the protective effect may be lost or significantly reduced. Oral desensitization is likely due to increased food-specific IgG4 and decreased food-specific IgE antibodies, and decreased reactivity of effector

cells (mast cells and basophils). In contrast, permanent oral tolerance is not affected by prolonged periods of food abstinence. The mechanism of persistent oral tolerance likely involves the initial development of regulatory T cells and immunologic deviation away from the proallergic  $T_H2$  response and, in later stages, anergy. The permanence of protection may be tested with intentional interruption of dosing for at least 4 to 8 weeks followed by a supervised oral food challenge.

### ***Subcutaneous Peanut Immunotherapy***

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The first evidence that immunotherapy may decrease the threshold of reactivity (desensitize) to a food allergen was provided by two controlled studies of subcutaneous peanut immunotherapy. In the initial study, 3 subjects treated with peanut extract had 67% to 100% symptom reduction during double-blind, placebo-controlled peanut challenges and a 2-log to 5-log reduction in endpoint skin prick skin test reactivity to peanut.<sup>20</sup> One placebo-treated subject completed the study and had no change in peanut challenge symptoms or skin prick test sensitivity to peanut. In a follow-up study of 12 subjects, 6 were treated with a maintenance dose of 0.5 mL of 1:100 wt/vol peanut extract for 12 months.<sup>21</sup> All treated subjects were able to ingest increased quantities of peanut during oral challenges and had decreased sensitivity on titrated peanut skin prick test, whereas untreated controls experienced no changes. Anaphylaxis after subcutaneous dose administration, however, occurred a mean of 7.7 times during 12 months in the 6 patients who received maintenance, with an average of 9.8 epinephrine injections per study subject. Only 3 subjects (50%) were able to achieve the maintenance dose due to adverse events. This important study demonstrated that injected peanut allergen could be successfully used to induce desensitization, but the significant risk for anaphylaxis precluded this treatment from being further evaluated in clinical studies.

### ***Birch Pollen Immunotherapy for the Pollen-Food Allergy Syndrome***

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Individuals sensitized to airborne pollen proteins may develop oral-pharyngeal pruritus from ingestion of raw fruits and vegetables that contain proteins homologous to the proteins in pollen. The classic pollen-food allergy syndrome (PFAS) or oral allergy syndrome is due to sensitization to the birch pollen major allergen, Bet v 1. Oropharyngeal symptoms occur on contact with the pollen cross-reactive apple allergen Mal d 1. Subcutaneous immunotherapy is an established treatment of pollen allergic rhinitis and, theoretically, could also benefit PFAS. An open trial of subcutaneous birch pollen immunotherapy in 49 adults with birch pollen allergic rhinitis and oral symptoms induced by raw apple reported a significant reduction (50%–95%) or complete resolution of apple-induced oral allergy symptoms ( $P < .001$ ) in 41 (84%) compared with no improvement in controls.<sup>22</sup> In a follow-up study, symptoms and skin test reactivity were compared after the 12-month immunotherapy course and 30 months after discontinuation of immunotherapy.<sup>23</sup> More than 50% of subjects still tolerated apple at the 30-month follow-up visit, although the majority showed a return of pretreatment sensitization by skin test. Subsequent clinical trials, in which oral allergy symptoms to apple were diagnosed with double-blind, placebo-controlled food challenges, confirmed a beneficial effect of subcutaneous birch pollen immunotherapy in a subset of subjects.<sup>24,25</sup> Similar findings were reported from an observational study of 16 adult subjects suffering from PFAS (hazelnut, walnut, lettuce, peach, and cherry) and plane tree pollen allergic rhinitis who were treated with plane tree pollen immunotherapy.<sup>26</sup> Doses of immunotherapy higher than those typically required to induce improvement in seasonal birch pollen rhinitis may be necessary to improve birch-related PFAS. The most beneficial effects on PFAS were observed in the studies that included adults

monosensitized to birch tree. In addition, the T-cell immune responses to birch pollen cross-reactive food allergens, such as apple (Mal d 1), hazelnut (Cor a 1), and carrot (Dau c 1), might be at least in part Bet v 1 independent. In that case, vaccines based on modified recombinant food allergens would represent a superior approach to the treatment of PFAS.<sup>27</sup> A few case reports highlighted the possibility of inducing allergy to cross-reactive food allergens in the course of subcutaneous birch pollen immunotherapy for the environmental allergens, such as development of allergy to snails during immunotherapy to dust mites or to raw fruits during immunotherapy to pollens.

### ***Oral Immunotherapy***

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The first report of successful OIT, in a boy with severe anaphylactic allergy to egg, was published in the early twentieth century.<sup>28</sup> OIT is currently being explored for cow's milk, peanut, and egg allergies. The oral route of administration takes advantage of cells and immune pathways involved in the induction of oral tolerance. Animal studies suggest that feeding high doses of an antigen causes nonresponsiveness due to anergy or deletion of antigen-specific T lymphocytes, whereas continuous feeding low doses of an antigen induces regulatory T cells.<sup>3,29</sup> In contrast, intermittent feedings or nonoral exposures (eg, cutaneous or inhalational) predispose to IgE sensitization and allergic symptoms after food ingestion.<sup>30</sup>

### **ORAL IMMUNOTHERAPY TRIALS**

During OIT, food is mixed in a vehicle and ingested in gradually increasing doses. The dose escalation occurs in a controlled setting; regular ingestion of tolerated doses during the buildup phase and a maintenance dose occurs at home. Early case series and uncontrolled trials provided evidence that a subset of food-allergic subjects could be desensitized to milk, egg, fish, fruit, peanut, and celery.<sup>31–34</sup> In some subjects who ultimately tolerated a maintenance dose, even for a significant period of time, allergic symptoms redeveloped if the food was not ingested on a regular basis, highlighting a concern that permanent tolerance was not achieved.<sup>35</sup>

In the first randomized trial of OIT, children with IgE-mediated cow's milk allergy (CMA) or hen's egg allergy were randomly assigned to OIT or elimination diet as a control group.<sup>36</sup> OIT treatment with fresh cow's milk or lyophilized egg white protein was performed daily at home. Children were re-evaluated by food challenge after a median of 21 months. Children in the OIT group were subsequently placed on an elimination diet for 2 months before a follow-up rechallenge to test for permanent oral tolerance. At follow-up challenge, 9 of 25 children (36%) showed permanent tolerance in the OIT group, 3 of 25 (12%) were tolerant with regular intake, and 4 of 25 (16%) were partial responders. In the control group, 7 of 20 children (35%) also developed tolerance over the study period. Although the rate of permanent tolerance was not different between the groups, some children were tolerant with regular intake and some were tolerant to a smaller maintenance dose (desensitized) and were protected from inadvertent exposures as they continued to ingest the daily dose of the food.

In the first placebo-controlled trial of OIT, 20 children with IgE-mediated milk allergy were randomized 2:1 to milk or placebo OIT.<sup>37</sup> The buildup occurred during an in-office day (initial dose, 0.4 mg of milk protein; final dose, 50 mg), followed by daily dosing at home with 8 weekly in-office dose increases to a maximum of 500 mg. Home daily maintenance doses were continued for 3 to 4 months. Double-blind, placebo-controlled milk challenges, skin prick tests, and milk protein serologic studies were performed before and after OIT. Nineteen patients, 6 to 17 years of age,

completed the treatment: 12 in the active group and 7 in the placebo group. The median milk cumulative dose inducing a reaction in both groups was 40 mg at the baseline challenge. After OIT, the median cumulative dose in the active treatment group was 5140 mg (range 2540–8140 mg), whereas all patients in the placebo group reacted at 40 mg ( $P = .0003$ ). Among 2437 active OIT doses and 1193 placebo doses, there were 1107 (45.4%) and 134 (11.2%) total reactions, respectively, most commonly local symptoms (**Table 4**). Milk-specific IgE levels did not change significantly in either group. Milk-IgG levels increased significantly in the active treatment group, with a predominant milk-IgG<sub>4</sub> level increase.

Safety and efficacy of OIT were investigated in 60 children with a history of severe milk-induced anaphylaxis and milk-specific IgE greater than 85 kU<sub>A</sub>/L, who reacted to less than or equal to 0.8 mL of milk during a baseline oral milk challenge.<sup>38</sup> Children were randomly divided into two groups: 30 children received OIT with a 10-day rush phase, including 3 to 10 daily doses up to 20 mL of undiluted milk in the hospital, followed by a slow dose-escalation phase at home (increasing by 1 mL every second day); the remaining 30 comparison subjects continued on a milk-free diet. After 1

**Table 4**  
**Benefits and risks of food oral immunotherapy**

| Success Rate <sup>a</sup>  | % of Treated Subjects                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Peanut                     | ~77 <sup>40</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Milk                       | ~37–70 <sup>37,38,43</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Egg                        | ~55 <sup>39</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Side Effects               | % of the doses of immunotherapy associated with side effects                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Peanut <sup>40,42,44</sup> | Buildup<br>Mild oral and pharyngeal symptoms: 69<br>Mild or moderate skin symptoms: 62<br>Mild to moderate nausea or abdominal pain: 44<br>Diarrhea/emesis: 21<br>Mild wheezing: 18<br>Maintenance<br>Upper respiratory symptoms: 29<br>Cutaneous: 24<br>Treatment of adverse reactions given for 0.7 of home doses,<br>2 subjects received one dose epinephrine                                                                                                                                                                                                                                                                                                                                                                 |
| Milk <sup>36,41</sup>      | Blinded, randomized, placebo-controlled study <sup>37</sup><br>Local (oral pruritus) median: 16% of doses per child<br>Gastrointestinal median: 2% of doses per child<br>Epinephrine was used in 0.2% of total doses; 4 doses of epinephrine<br>were used to treat 4 subjects: 2 doses during buildup and 2 doses<br>during home maintenance<br>Open-label home study <sup>43</sup><br>2.5%–96.4% of Doses per subject in the first 3 months compared<br>with 0%–79% in the subsequent months<br>Total percentages of doses with reactions:<br>Local reactions (oral pruritus): 17<br>Gastrointestinal: 3.7<br>Respiratory: 0.9<br>Cutaneous: 0.8<br>Multisystem: 5.5<br>Epinephrine was used for 6 reactions: 0.2% (4 subjects) |

<sup>a</sup> Defined as ability to ingest the significant amount food on a regular basis for at least 6 months which is equivalent to desensitized state.

year, 11 of 30 (36%) subjects in the OIT group were able to ingest a daily dose of milk equal or greater than 150 mL; 16 subjects (54%) were able to ingest from 5 mL to less than 150 mL. Three children (10%) were unable to complete the OIT because of the frequent adverse reactions. In the comparison group, all 30 children reacted to less than 5 mL of milk during the repeated oral food challenge at 12 months. Adverse reactions were common in both groups but no child had severe anaphylaxis. During the rush phase, intramuscular epinephrine was administered 4 times; 1 dose per child in 4 children. During the home phase, 2 children required treatment, including epinephrine in the emergency department.

### ***Egg OIT***

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Another study reported preliminary results on 7 children with nonanaphylactic egg allergy treated with egg OIT.<sup>39</sup> These children underwent a 24-month egg OIT protocol involving modified rush buildup and maintenance phases. Double-blind, placebo-controlled food challenges were performed at the end of the study and all children tolerated significantly more egg protein than at study onset. Two subjects remained tolerant to egg after a period of strict egg avoidance. Egg-specific IgG concentrations increased significantly, whereas there was no significant change in egg-specific IgE concentrations.

### ***Peanut OIT***

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Peanut OIT trials conducted in young children with peanut allergy have attracted significant attention.<sup>40,41</sup> In a US study, 39 subjects were enrolled (64% boys). The median age at enrollment was 57.5 months (range, 12–111 months).<sup>40</sup> All children completed the initial day escalation phase, during which the starting dose of 0.1-mg peanut protein was doubled every 30 minutes, up to 50 mg. During the buildup phase, children ingested peanut flour with other safe food every day. Doses were increased by 25 mg every 2 weeks until 300 mg was reached. During the maintenance phase, the dose of 300 mg was continued daily until the follow-up food challenge was performed. After the food challenge, the daily dose was increased to 1800 mg. Children were evaluated every 4 months while on continued maintenance dosing (a total of 36 months). Ten (25%) children subsequently withdrew after the initial day escalation phase. Six discontinued for personal reasons, including transportation issues, parental anxiety, and failure to perform home dosing. The other 4 subjects discontinued because of allergic reactions to the OIT that did not resolve with continued treatment or dose reduction. Three had gastrointestinal complaints, and 1 had symptoms of asthma. Twenty-nine subjects completed all 3 phases of the study and peanut challenges.

During the initial day escalation, 36 patients (92%) experienced some symptoms; most common were upper respiratory symptoms, with 27 patients (69%) reporting mild sneezing/itching and mild laryngeal symptoms. No patients experienced severe upper respiratory or laryngeal symptoms; 17 patients (44%) reported mild to moderate nausea or abdominal pain; 8 patients (21%) had diarrhea/emesis; and 24 subjects (62%) had mild or moderate skin symptoms. A total of 6 patients experienced chest symptoms during the initial escalation day; all 6 had mild wheezing, and 2 progressed to moderate wheezing. Three of the subjects with chest symptoms during the initial day escalation also had a previous diagnosis of asthma. During the final food challenge, 27 of the 29 children who completed the protocol ingested 3.9 g of peanut flour (approximate equivalent of 11 peanut kernels). By 6 months, titrated skin prick tests and activation of basophils decreased significantly. Peanut-specific IgE antibody concentrations decreased by 12 to 18 months whereas peanut-specific IgG4 increased significantly.

Among the children who participated in the trial of peanut OIT, 20 of 39 completed all phases of the study.<sup>40,42</sup> Risk of mild wheezing was 15% (6/39) during the initial escalation. The risk of any symptoms after the buildup phase doses was 46%, with a risk of 29% for upper respiratory tract and 24% for skin symptoms. The risk of an adverse reaction with any home dose was 3.5%: upper respiratory tract (1.2%) and skin (1.1%) symptoms. Treatment was given for 0.7% of home doses. Two subjects received epinephrine after 1 home dose each. Allergic reactions during home dosing were significantly more common in the milk OIT, from 2.55 to 96.4% of doses per subject in the first 3 months compared with 0% to 79.8% in the subsequent 3 months.<sup>43</sup> Local and multisystem reactions decreased whereas all other reactions remained unchanged during the maintenance phase of OIT. Systemic reactions occurred at previously tolerated doses in the setting of exercise or viral illness. As recently highlighted, the risk of an allergic reaction to a previously tolerated dose of food is associated with physical exertion after dosing, dosing on empty stomach, dosing during menses, concurrent febrile illness, and suboptimally controlled asthma (see **Table 4**).<sup>36,43,44</sup>

### SUBLINGUAL IMMUNOTHERAPY

SLIT with food represents an alternative approach to desensitization or oral tolerance and was first reported using fresh kiwifruit pulp extract in a 29-year-old woman with a history of kiwifruit-induced anaphylaxis.<sup>45</sup> The extract or kiwifruit cube was kept under the tongue for 1 minute before swallowing (combined SLIT and OIT). Five years into kiwifruit-modified SLIT, treatment was interrupted for 4 months and then resumed without any problems.<sup>46</sup>

In a randomized, double-blind, placebo-controlled trial, adults with hazelnut allergy (54.5% with a history of oral allergy symptoms) confirmed by double-blind placebo-controlled food challenge were randomly assigned to 1 of 2 groups, hazelnut immunotherapy ( $n = 12$ ) or placebo ( $n = 11$ ).<sup>47</sup> Subjects kept the hazelnut extract solution in the mouth for at least 3 minutes and then spat it out. All treated subjects reached the planned maximum dose with a 4-day rush protocol, followed by a daily maintenance dose (containing 188.2  $\mu\text{g}$  of Cor a 1 and 121.9  $\mu\text{g}$  of Cor a 8, major hazelnut allergens). Systemic reactions were observed in 0.2% of the total doses administered during the rush buildup phase and were treated with oral antihistamines. Local reactions, mainly immediate oral pruritus, were observed in 7.4% (109 reactions/1466 doses). Four patients in the active group reported abdominal pain several hours after dosing on one occasion each during the buildup phase. All local reactions during the maintenance phase were limited to oral pruritus and occurred in a single patient. After 5 months of SLIT, the mean threshold dose of ingested hazelnut-provoking allergic symptoms increased from 2.3 g to 11.6 g in the active group ( $P = .02$ ) compared with 3.5 g to 4.1 g in placebo (non-significant). Almost 50% of treated subjects tolerated the highest dose (20 g) of hazelnut during follow-up, double-blind, placebo-controlled food challenge compared with 9% in the placebo group. Levels of serum hazelnut-specific IgG4 antibody and total serum interleukin (IL)-10 increased only in the active group, but there were no differences in hazelnut-specific IgE antibody levels preimmunotherapy and postimmunotherapy.

Another study evaluated SLIT in 8 children with CMA.<sup>48</sup> A day after an initial positive oral milk challenge, children started SLIT with 0.1 mL of milk for the first 2 weeks, increasing by 0.1 mL every 15 days until 1 mL per day was given. Milk was kept in the mouth for 2 minutes and then discharged. Seven subjects completed the protocol; one subject withdrew due to oral symptoms. After 6 months of treatment, the threshold dose of milk increased from a mean of 39 mL at baseline to 143 mL ( $P < .01$ ).

Preliminary data on OIT and SLIT are encouraging; however, at this time, these treatments are considered experimental and additional studies must address many issues, including the optimal dose; ideal duration of OIT/SLIT; degree of protection, safety, and efficacy for different ages; severity and type of food allergies responsive to treatment; and the need for patient protection during home administration. In view of the recent reports of reactions to the tolerated doses of OIT at home, it may be necessary to hold doses during acute febrile illness, avoid exercise within 2 hours after dosing, and take the daily OIT dose with a meal or snack.<sup>44</sup> Rhinitis and asthma should be maintained under optimal control. Finally, because a subset of children with food allergies develops tolerance spontaneously, future studies must address diagnostic tests that distinguish among those with transient and persistent food allergies to identify the subjects who will benefit from therapy.

### EPICUTANEOUS IMMUNOTHERAPY

Epicutaneous patches that solubilize the allergen by perspiration and disseminate it into the thickness of the stratum corneum represent an alternative delivery route of immunotherapy.<sup>49</sup> Epicutaneous delivery is less invasive than subcutaneous injection and may have a lower risk for systemic reactions than subcutaneous, oral, or sublingual food allergen delivery. A proof-of-concept study on the efficacy of EPIT on intact skin was done in mice sensitized to aeroallergens or food allergens.<sup>50</sup> Mice were sensitized to pollen ( $n = 18$ ), house dust mite ( $n = 24$ ), ovalbumin ( $n = 18$ ), or peanut ( $n = 18$ ) and allocated to 1 of 3 groups: EPIT, subcutaneous immunotherapy, and no treatment. In addition, control animals not sensitized and not treated were followed as a fourth group. After 8 weeks of treatment, lung function testing by plethysmography was performed after aerosol provocation with appropriate allergens and showed reduced airway responsiveness to methacholine in EPIT mice. IgG2a for pollen, house dust mite, ovalbumin, and peanuts was significantly increased in the EPIT group versus not treated mice. In mice sensitized to the 4 allergens tested, EPIT was as efficacious as subcutaneous immunotherapy, considered the reference immunotherapy. EPIT may represent a more convenient route with a superior safety profile compared with subcutaneous immunotherapy, OIT, or SLIT.

In a pilot study, 18 children (mean age 3.8 years, range 10 months to 7.7 years) with CMA were randomized 1:1 to receive active EPIT or placebo.<sup>51</sup> CMA was confirmed by a clinician-supervised oral food challenge at baseline and the cumulative tolerated dose of milk was established. Children received three 48-hour applications (1 mg skimmed milk powder or 1 mg glucose as placebo) via the skin patch per week for 3 months. Treated children showed a trend toward increased cumulative tolerated dose at the follow-up oral milk challenge after 3 months of EPIT, from a mean 1.8 mL at baseline to 23.6 mL at 3 months. The mean cumulative tolerated dose did not change in the placebo-treated group. There were no significant changes in cow's milk-specific IgE levels from baseline to 3 months in either group. The most common side effects were local pruritus and eczema at the site of application. There were no severe systemic adverse reactions; however, one subject in the active group had repeated episodes of diarrhea after EPIT with milk. Reports from earlier mouse studies have demonstrated increased potential for the development of IgE sensitization to peanut via the epicutaneous route compared with ingestion, raising concerns as to whether epicutaneous delivery might worsen food allergy.<sup>30</sup> It is impossible to fully understand the effect of EPIT on milk allergy from this small pilot study due to the small sample size and short duration of the study as well as limited information about immunologic parameters. This preliminary report suggests, however, that further

investigation of the novel epicutaneous antigen delivery for food allergy immunotherapy is warranted.

### DIET CONTAINING EXTENSIVELY HEATED COW'S MILK AND EGG

Two large clinical trials investigated the tolerance of extensively heated (baked into other products) milk and egg in children with milk ( $n = 100$ ) and egg ( $n = 117$ ) allergy.<sup>52,53</sup> Thermal processing largely destroys IgE-binding epitopes but not sequential IgE-binding epitopes (see **Fig. 1**). Prior studies determined that children with persistent milk and egg allergy generated IgE antibodies directed primarily against sequential epitopes of egg ovomucoid and milk casein.<sup>8,9</sup> These observations suggested that at least a subset of children might tolerate extensively heated (eg, baked) products with milk and egg. In each study, more than 80% of children were tolerant to milk and egg baked into muffins and waffles during an initial oral challenge and incorporated these foods into their daily diet at home. Children were being followed every 3 to 6 months and tolerated the baked diet well. There was no increase in acute allergic reactions and no increase in severity of underlying atopic diseases, such as asthma, atopic dermatitis, or eczema. There was no increase in the intestinal permeability of carbohydrate markers (lactulose and mannitol) over the first year on the diet and children had no growth impairment. Commercially available tests for food-specific IgE levels did not reliably identify subjects tolerant to extensively heated milk and egg, and oral food challenge was indispensable. Children tolerant to unheated milk, however, had the lowest basophil reactivity to milk whereas children who reacted to extensively heated milk had significantly higher basophil reactivity to stimulation with milk protein.<sup>54</sup> In the milk study, the majority of children who reacted to extensively heated milk had milk-specific IgE antibody levels greater than 35 kU<sub>A</sub>/L (UniCAP, Phadia) and, therefore, in a subsequent study, subjects with milk-specific IgE antibody levels greater than 35 kU<sub>A</sub>/L were excluded. In the milk study, severe reactions that required treatment with epinephrine occurred only in children who reacted to the extensively heated milk products. All of the subjects who tolerated the extensively heated milk products and subsequently reacted to unheated milk had milder reactions and none received epinephrine. This suggests that tolerance to extensively heated milk products is a marker of a milder milk allergy that is likely to be outgrown. In contrast, in the egg study, there were equal proportions of children who received epinephrine during the heated and unheated egg challenges.

The immunologic changes observed during the ingestion of baked goods with milk and egg included increasing food-specific IgG4 antibodies, decreasing wheal sizes from prick skin tests, and no consistent changes in the food-specific IgE antibodies. There was a significantly higher percentage of casein-specific regulatory T cells in extensively heated milk-tolerant children compared with children with allergy to extensively heated milk. A higher frequency of casein-specific regulatory T cells correlated with a phenotype of mild clinical disease and favorable prognosis.<sup>55</sup>

These findings suggest that large subsets of children with milk and egg allergy might include extensively heated products in their diets. Furthermore, the immunologic changes induced by the diet containing baked milk and egg products are similar to the changes observed during OIT trials. Tolerance to extensively heated milk and egg might identify subjects with favorable prognosis. The diet containing extensively heated milk and egg might represent a safer and more natural approach to oral immunomodulation. Until further studies have been completed to establish the overall safety and efficacy of this method, however, baked milk and egg diet should be approached with caution and introduction of heated foods done under physician supervision.

## IMMUNOTHERAPY WITH RECOMBINANT ENGINEERED FOOD PROTEINS

Alteration of the IgE antibody-binding sites (epitopes) can decrease the risk of an acute allergic reaction during immunotherapy. Point mutations introduced by site-directed mutagenesis in the known IgE epitopes of major food allergens and polymerization of proteins result in decreased IgE binding during immunotherapy. In vivo efficacy of engineered recombinant peanut proteins was first tested in a mouse model of peanut anaphylaxis.<sup>56,57</sup> Mice were sensitized to whole peanut and then desensitized by intranasal administration of engineered recombinant Ara h 2 (3 doses per week for 4 weeks). Desensitization with the engineered recombinant Ara h 2 protein significantly decreased severity of anaphylactic reactions after oral peanut challenge compared with a control group.

Modified food allergens may be combined with bacterial adjuvants to further reduce food-specific IgE production. Initially, heat-killed *Listeria monocytogenes* combined with engineered peanut allergens (mAra h 1–3) was shown effective in preventing peanut anaphylaxis.<sup>58</sup> Safety concerns about using whole-cell pathogenic bacteria in humans were raised, however, due to the risk of inducing adverse inflammatory reactions. In subsequent studies, a nonpathogenic strain of *Escherichia coli* was used as an adjuvant and vaccine was delivered orally and rectally. Oral delivery was not effective, presumably due to breakdown of the peanut-containing *E. coli*. Peanut-allergic C3H/HeJ mice treated with 3 different doses of heat-killed *E. coli* expressing modified proteins Ara h 1–3 (HKE-MP123) per rectum had reduced severity of anaphylaxis ( $P < .01$ ) compared with the sham-treated group.<sup>59</sup> Only the medium-dose and high-dose HKE-MP123-treated mice, however, remained protected for up to 10 weeks after treatment. Peanut-specific IgE levels were significantly lower in all HKE-MP123-treated groups ( $P < .001$ ); they were most reduced in the high-dose HKE-MP123-treated group at the time of each challenge. A phase I clinical safety study is currently enrolling adult subjects with peanut allergy. In the future, probiotic bacteria might be used as adjuvants to avoid the concerns of excessive T-helper cell type 1 ( $T_H1$ ) stimulation by killed pathogenic bacteria.

## PROBIOTICS FOR PREVENTION AND TREATMENT OF FOOD ALLERGY

The concept of probiotic foods was introduced more than a century ago by Fuller. Probiotics are live bacteria or their components that have beneficial effect on the health of the host presumably by improving intestinal microbial balance. The major sources of probiotics are dairy products that contain *Lactobacillus* and *Bifidobacterium* species.

Probiotics generate increasing interest as a potential approach to food allergy prevention and therapy (Table 5). In one trial, 159 expectant mothers who had at least one first-degree relative (or partner) with history of atopic disease (atopic dermatitis, asthma, or allergic rhinitis) were randomized to either receive *Lactobacillus* GG supplementation or placebo.<sup>60</sup> Treatment was continued during breastfeeding and was given to the infants for 6 months, starting on the first day of life. At age 2 years, 23% of children had atopic dermatitis in the probiotic-treated group compared with 46% in the placebo group ( $P = .008$ ), relative risk of 0.51 (95% CI, 0.32–0.84), indicating that *Lactobacillus* GG had some effect on the prevention of early atopic disease in infants at high risk. This study, however, raised several questions. The prevalence of atopic dermatitis in the placebo-treated group was very high but the severity of atopic dermatitis was very low (geometric mean scoring atopic dermatitis [SCORAD] index of 10.4 out of maximum 103) and not different from the probiotic-treated group (geometric mean SCORAD index of 9.8). Furthermore, there was no difference between the two study groups in any of the objective markers, such as the prevalence

**Table 5**  
**Probiotics for food allergy prevention and treatment**

|                                                                                                                                                      | Mechanism of Action                                                                                                                                                                                                                                      | Effects                                                                                                                                                                                                                                                            | Comments                                                                                                                                                                                                                                                                            |
|------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Prevention</b>                                                                                                                                    |                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                     |
| <i>Lactobacillus</i> GG randomized human trial (N = 159), mother supplemented before delivery and infants supplemented for 6 months <sup>60,61</sup> | Unclear; there was no difference between the two study groups in number of positive prick skin tests to selected food and environmental allergens, total serum IgE as well as serum allergen-specific IgE levels to milk, egg, cat, and house dust mite. | At age 2 years, 23% of children had atopic dermatitis in the probiotic-treated group compared with 46% in the placebo group ( $P = .008$ ); the protective effect persisted up to 7 years of age.                                                                  | The prevalence of atopic dermatitis in the placebo-treated group was very high but the severity of atopic dermatitis was very low (geometric mean SCORAD index of 10.4 out of maximum 103) and not different from the probiotic-treated group (geometric mean SCORAD index of 9.8). |
| Cow's milk-based formula with prebiotics <sup>62</sup>                                                                                               | Prebiotics enhance gut colonization with healthy commensal (probiotic) bacteria.                                                                                                                                                                         | Cumulative incidence of atopic dermatitis at 1 year of age was significantly lower in the prebiotic group (5.7%) than in the control group (9.7%, $P = .04$ ).                                                                                                     | There was no effect on the IgE-sensitization to egg white and cow's milk. The number needed to prevent 1 case of atopic dermatitis by supplementation of prebiotics was 25 infants.                                                                                                 |
| <i>Lactococcus lactis</i> transfected with murine IL-10 <sup>67</sup>                                                                                | Decreased serum IgE and IgG1, increased IgA in the gut, increased gut and serum IL-10.                                                                                                                                                                   | Pretreatment of young mice before sensitization with $\beta$ -lactoglobulin in the presence of cholera toxin protected against anaphylaxis on the oral food challenge.                                                                                             | This approach was only tested in the mouse model; however, the concept of probiotic bacteria may be applied to delivery of engineered allergens in human studies.                                                                                                                   |
| <b>Treatment</b>                                                                                                                                     |                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                     |
| <i>Lactobacillus rhamnosus</i> GG and <i>Bifidobacterium lactis</i> <sup>63</sup>                                                                    | Symptomatic improvement was associated with the increase in IgA and the suppression of TNF- $\alpha$ synthesis.                                                                                                                                          | 2-Month treatment with <i>Lactobacillus rhamnosus</i> GG and <i>Bifidobacterium lactis</i> in addition to a milk elimination diet decreased the severity of atopic dermatitis symptoms in infants and young children with established cow's milk hypersensitivity. |                                                                                                                                                                                                                                                                                     |

of positive prick skin tests to selected food and environmental allergens, total serum IgE, and serum allergen-specific IgE levels to milk, egg, cat, and house dust mite. Nevertheless, the 7-year follow-up data confirmed the long-lasting protective effect of early *Lactobacillus* GG supplementation.<sup>61</sup> Risk of eczema was significantly reduced in the *Lactobacillus* GG group compared with the placebo group, odds ratio 0.58 (95% CI, 0.35–0.94;  $P = .03$ ). The effect of prebiotics-oligosaccharides shown to promote probiotic colonization of the gastrointestinal tract on primary prevention of atopic dermatitis was investigated in 830 healthy term infants at low risk for atopy (no history of allergic disease, such as atopic dermatitis, asthma, or hay fever, in any parent or sibling).<sup>62</sup> Infants were randomized 1:1 to feeding with cow's milk-based formula containing prebiotics (a mixture of neutral and pectin-derived acidic oligosaccharides) or with cow's milk-based formula without prebiotics. Up to the first birthday, atopic dermatitis occurred in 5.7% infants in the prebiotic group compared with 9.7% infants in the control group ( $P = .04$ ).

In another study, 2-month treatment with *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* in addition to a milk elimination diet decreased the severity of atopic dermatitis symptoms infants and young children with established cow's milk hypersensitivity.<sup>63</sup> Symptomatic improvement was associated with the increase in IgA and the suppression of tumor necrosis factor (TNF)- $\alpha$  synthesis. The exact mechanisms by which probiotics may affect atopic disease remain speculative. Possible mechanisms of probiotic immunomodulation include increased synthesis of IgA and IL-10, suppression of TNF- $\alpha$ , inhibition of casein-induced T-cell activation and circulating soluble CD4, and Toll-like receptor 4 signaling.<sup>64–66</sup>

### **Probiotic Bacteria Expressing IL-10**

*Lactococcus lactis* was transfected to secrete murine IL-10 and then given to young mice before oral sensitization with  $\beta$ -lactoglobulin, a whey protein in cow's milk.<sup>67</sup> Symptoms during oral challenge and serum and fecal  $\beta$ -lactoglobulin-specific antibody concentrations were measured. Antibody titers were correlated with numbers of cells secreting IL-10 in the spleen and Peyer patches. Pretreatment with IL-10-transfected *Lactococcus lactis* reduced anaphylaxis symptoms and inhibited  $\beta$ -lactoglobulin-specific serum IgE and IgG1 concentrations. It also increased the production of  $\beta$ -lactoglobulin-specific IgA in the gut. These results suggest that a probiotic bacteria engineered to deliver IL-10 in the gut might be able to decrease food-induced anaphylaxis and provide an option to prevent IgE-type sensitization in a murine model of milk allergy. These findings need to be validated in human studies for prevention of food allergy.

### **SUMMARY**

Food allergy is an increasingly prevalent problem in westernized societies. A variety of promising immunomodulatory approaches are under investigation for treatment and prevention of food allergy.

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