Food Allergy: Recent Advances in Pathophysiology and Treatment

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Abstract
Food allergies, defined as an adverse immune response to food proteins, affect as many as 6% of young children and 3%–4% of adults in westernized countries, and their prevalence appears to be rising. In addition to well-recognized acute allergic reactions and anaphylaxis triggered by IgE antibody–mediated immune responses to food proteins, there is an increasing recognition of cell-mediated disorders such as eosinophilic gastroenteropathies and food protein–induced enterocolitis syndrome. We are gaining an increasing understanding of the pathophysiology of food allergic disorders and are beginning to comprehend how these result from a failure to establish or maintain normal oral tolerance. Many food allergens have been characterized at a molecular level, and this knowledge, combined with an increasing appreciation of the nature of humoral and cellular immune responses resulting in allergy or tolerance, is leading to novel therapeutic approaches. Currently, management of food allergies consists of educating the patient to avoid ingesting the responsible allergen and initiating therapy if ingestion occurs. However, numerous strategies for definitive treatment are being studied, including sublingual/oral immunotherapy, injection of anti-IgE antibodies, cytokine/anticytokine therapies, Chinese herbal therapies, and novel immunotherapies utilizing engineered proteins and strategic immunomodulators.
INTRODUCTION

Food allergy/hypersensitivity disorders are defined as adverse immune responses to food proteins. This characterization distinguishes these disorders from many adverse food reactions that have nonimmune etiologies (1). Food allergic disorders include acute, possibly life-threatening allergic reactions, as well as chronic debilitating diseases such as atopic dermatitis or eosinophilic gastroenteropathies. An accurate epidemiological assessment of the burden of food allergy is hampered by the lack of population-based studies incorporating the gold-standard diagnostic method, a double-blind placebo-controlled oral food challenge. A meta-analysis focusing on allergy to milk, egg, peanut, and seafood, which included studies that incorporated oral food challenges, showed an overall approximate prevalence of 3.5% (2). There is a well-supported notion that food allergy has increased in the past 10–15 years, particularly in developed countries. For example, studies in the United States and United Kingdom indicate that peanut allergy has increased about twofold among children, with >1% currently affected (3–5).

The current treatment of food allergic disease is based on identification and avoidance of triggering foods, and reactionary therapy such as injection of epinephrine in the event of anaphylaxis. Modest gains have been made in diagnosis, prevention, and management (6). In this review, we focus on insights in disease immunopathophysiology and the translation of new knowledge into therapeutic modalities being evaluated now and in the near future.

MECHANISMS OF FOOD ALLERGIC DISORDERS

The diverse clinical manifestations of food allergy result in part from different immune mechanisms, target organ responses, and characteristics of triggering proteins (1). Conceptually, it is useful to categorize the immune mechanisms according to three general types (Table 1): immunoglobulin E (IgE) antibody-dependent diseases, cell-mediated disorders (without detectable IgE antibodies), and disorders with mixed IgE and non-IgE mechanisms. The specificity and degree of immune response also affects disease expression. For example, many individuals have detectable food-specific IgE but do not experience clinical reactions when they ingest that food (1). However, increasing quantities of food-specific IgE in the serum (and presumably on mast cells and basophils) are associated with increasing risk of clinical allergy (7). Furthermore, individuals at greatest risk for severe reactions to peanut, for example, demonstrate patterns of IgE recognition to an increased number of peanut protein IgE-binding (allergenic) epitopes, or binding to specific proteins in peanut (8–10), presumably mediating increased efficiency of activating effector cells.

Additional host factors influence outcomes. In a study of patients who experienced fatal and nonfatal peanut-induced anaphylaxis, elevated platelet-activating factor (PAF) and decreased PAF acetylhydrolase were associated with increased severity or fatal reactions, raising the possibility that failure of PAF acetylhydrolase to inactivate PAF contributes to reaction severity (11). Target organ sensitivity also affects outcomes. For example, persons with asthma are at higher risk to experience severe or fatal anaphylaxis (12), presumably because of heightened target organ responsiveness.

The characteristics of triggering proteins also affect disease expression. Proteins that are easily degraded by heat and digestion, including many of the proteins in fruits, are unlikely to trigger severe reactions. In contrast, stable proteins, such as seed storage proteins in nuts and seeds, are more likely to trigger systemic allergic reactions (13).

Food allergy is at least in part genetically determined. Peanut allergy, for example, is about tenfold more likely to occur in a child with a sibling who is peanut allergic compared to the general population risk; however, specific genes have not been identified (14). Recent epidemiological studies also identify potential environmental influences on immune function favoring
Table 1  Immunopathology of selected food allergic disorders

<table>
<thead>
<tr>
<th>Immunopathology (presentation on food exposure)</th>
<th>Disorder</th>
<th>Key features</th>
<th>Additional immunopathology</th>
<th>Common trigger foods</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE antibody dependent (acute onset)</td>
<td>Urticaria/angioedema</td>
<td>Wheal and flare, edema. Triggered by ingestion or by direct skin contact. Food commonly causes acute (20%) but rarely chronic (2%) urticaria</td>
<td></td>
<td>Multiple foods</td>
</tr>
<tr>
<td></td>
<td>Oral allergy syndrome (pollen-food-related)</td>
<td>Pruritus, mild edema confined to oral cavity</td>
<td>Sensitization to pollen proteins by the respiratory route results in IgE that binds certain homologous, typically labile, food proteins (in certain fruits/vegetables)</td>
<td>Raw fruit/vegetables</td>
</tr>
<tr>
<td></td>
<td>Rhinitis, asthma</td>
<td>These symptoms may accompany a food-allergic reaction but are rarely isolated or chronic symptoms</td>
<td></td>
<td>May be triggered by inhalation of aerosolized food protein (e.g., boiling milk, peanut flour) or by ingestion</td>
</tr>
<tr>
<td></td>
<td>Anaphylaxis</td>
<td>Rapidly progressive, multiple organ system reaction can include cardiovascular collapse</td>
<td>Massive release of mediators, though mast cell tryptase not always elevated</td>
<td>Peanuts, tree nuts, fish, shellfish, seeds, and milk among others</td>
</tr>
<tr>
<td></td>
<td>Food-associated, exercise-induced anaphylaxis</td>
<td>Food triggers anaphylaxis only if ingestion followed temporally by exercise</td>
<td>Exercise is presumed to alter gut absorption and/or allergen digestion</td>
<td>Wheat, shellfish, celery most commonly described</td>
</tr>
<tr>
<td>IgE antibody-associated/cell-mediated (delayed-onset/chronic)</td>
<td>Atopic dermatitis</td>
<td>Associated with food in ~35% of children with moderate to severe rash</td>
<td>May relate to homing of food-responsive T cells to the skin</td>
<td>Egg, milk, wheat, soy, among others</td>
</tr>
<tr>
<td></td>
<td>Eosinophilic gastroenteropathies</td>
<td>Symptoms vary with site(s)/degree of eosinophilic inflammation. Esophageal: dysphagia, pain. Generalized: ascites, weight loss, edema, obstruction</td>
<td>Mediators that home and activate eosinophils play a role, e.g., eotaxin, IL-5</td>
<td>Multiple foods</td>
</tr>
<tr>
<td>Cell-mediated (delayed-onset/chronic)</td>
<td>Dietary protein enterocolitis</td>
<td>Primarily affects infants. Chronic exposure: emesis, diarrhea, poor growth, lethargy. Re-exposure after restriction: emesis, diarrhea, hypotension (15%) 2 h following ingestion</td>
<td>Increased TNF-α response, decreased response to TGF-β</td>
<td>Cow’s milk, soy, grains</td>
</tr>
<tr>
<td></td>
<td>Dietary protein proctitis</td>
<td>Mucus-laden, bloody stools in infants</td>
<td></td>
<td>Cow’s milk via breast feeding</td>
</tr>
</tbody>
</table>
Oral allergy syndrome or pollen-food-related syndrome: allergy to a pollen protein results in symptoms following ingestion of a homologous food protein in certain raw fruits or vegetables. Allergic responses, including reduced exposures to bacteria and infections (the “hygiene hypothesis”), a rise in consumption of omega-6 and decreased consumption of omega-3 polyunsaturated fatty acids, reduced dietary antioxidants, and excess or deficiency of vitamin D (15). The complex interactions of host immune responses (including when and how oral tolerance is abrogated), food protein biochemistry, and various environmental and genetic influences set the stage for food allergic disease outcomes, but they also present multiple opportunities to interrupt the process and to develop effective therapies.

Mechanisms of Allergy and Tolerance
The gastrointestinal mucosal immune system must simultaneously battle pathogens, recognize and ignore harmless food proteins, and allow colonization with commensal bacteria. Disruption of this fine balance alters a normal state of oral tolerance to foods, possibly resulting in allergy. Oral tolerance may be breached directly during ingestion, or it may be bypassed altogether by presentation of proteins by alternative routes, such as via the respiratory tract or skin. In oral allergy syndrome, also known as pollen-food-related syndrome, oral tolerance is bypassed because sensitization occurs through the respiratory route (16). For example, respiratory sensitization to the birch pollen protein Bet v 1 may result in oral pruritus when patients eat raw apples because of cross-reactivity to a homologous apple protein, Mal d 1, which is otherwise not a strong oral allergen or tolerogen because it is easily degraded.

Evidence is accumulating that nonoral exposure to stable proteins may also provoke allergy, especially if oral tolerance was not already established. Murine models demonstrate that epicutaneous application of food proteins may result in sensitization leading to systemic allergy following oral exposure (17, 18). Human data also suggest that skin exposure may be sensitizing. In a population-based study, Lack et al. (19) found no evidence of increased risk of peanut allergy related to maternal peanut ingestion during pregnancy or lactation, but peanut allergy was associated with the use of infant skin creams containing peanut oil (odds ratio, 6.8) on children with atopic dermatitis, which has been associated with an increased risk of food allergy (19, 20). The loss of skin barrier provides a portal for sensitization to food allergens in the environment and is increasingly being considered a potential route by which food allergens may evade oral tolerance (15).

Oral tolerance depends on an intact and immunologically active gastrointestinal barrier (1, 21). This barrier includes the epithelial cells joined by tight junctions and a thick mucus layer, as well as luminal and brush border enzymes, bile salts, and extremes of pH, which combine to render antigens less immunogenic. In addition, innate (natural killer cells, polymorphonuclear leukocytes, macrophages, epithelial cells, and toll-like receptors) and adaptive immunity (intraepithelial and lamina propria lymphocytes, Peyer’s patches, SlgA, and cytokines) provide an active barrier to foreign antigens. Food allergy is more common in infants and often resolves even though there are signs of continued immune response, such as specific IgE antibodies. One explanation for this observation is that the immaturity of various components of the gut barrier—for example, an initially higher gastric pH—may reduce the efficiency of the infant mucosal barrier. Recent clinical observations and murine models indicate that treatment with antacid medications increases the risk of sensitization to ingested foods (22).

Antigen-presenting cells, including intestinal epithelial cells and dendritic cells, and regulatory T cells play a central role in oral tolerance (21, 23, 24). Several regulatory T cells have been associated with intestinal immunity: Th3 cells, a population of CD4+ cells that secrete TGF-β; Tr1 cells, cells that secrete IL-10; CD4+CD25+ regulatory T cells; CD8+ suppressor T cells; and gamma-delta T cells. Intestinal epithelial cells can process luminal antigen and present it to T cells on a class II major histocompatibility complex (MHC) but lack a “second signal,” suggesting their potential
to play a major role in induction of tolerance to food antigens as nonprofessional antigen-presenting cells. Dendritic cells residing within the lamina propria and noninflammatory environment of Peyer’s patches express IL-10 and IL-4, which favor tolerance (25).

Properties of antigens, dose of allergen, and frequency of exposure also influence tolerance induction. Murine models indicate that “high-dose” tolerance involves deletion of effector T cells, whereas “low-dose” tolerance is mediated by activation of regulatory T cells with suppressor functions (21). It has been suggested that T cells primed in the local mucosal environment lead to tolerance induction, whereas T cells primed in the mesenteric lymph nodes, from antigen reaching the node either in the lymph or carried by circulating dendritic cells, differentiate and travel to the mucosa where they induce local immune responses (23). Additional details about immune tolerance were recently reviewed (26).

Commensal gut flora also play a role in oral tolerance, as suggested by the observation that mice raised in a germ-free environment fail to develop normal tolerance (27). Mice treated with antibiotics, or lacking toll-like receptors recognizing bacterial lipopolysaccharides, were more prone to developing peanut allergy when exposed to a sensitizing regimen of peanut than were wild-type controls (28). Population-based observational studies relating atopic dermatitis to stool flora patterns, and interventional studies of probiotics, suggest a potential for allergy prevention by creating a tolerogenic bacterial milieu, although clinical studies are conflicting (29).

Allergic responses result from dysregulation in the immune network. The transcription factor FOXP3 is required to generate CD4+CD25+ regulatory T cells, and mutations in the FOXP3 gene result in a deficiency of these cells, which in turn leads to a syndrome that includes severe food allergies and atopic dermatitis (30). Karlsson et al. (31) explored a role for these cells in food allergy by evaluating children who did or did not resolve their cow’s milk allergy. Children who became milk tolerant had higher frequencies of circulating CD4+CD25+ T cells and decreased in vitro proliferative responses of peripheral blood mononuclear cells (PBMCs) to milk protein. When CD25+ cells were depleted in vitro in the samples from tolerant children, there was a fivefold increase in proliferation of PBMCs. In a murine model, van Wijk et al. (32) depleted CD4+CD25+ T cells at different points in tolerizing and sensitizing protocols to peanut. There was a loss of ability to induce low-dose tolerance to peanut proteins, but a long-term exposure protocol did not result in sensitization. However, in sensitized mice, depletion of T regulatory cells during oral exposure resulted in stronger allergic responses. These studies strongly suggest that CD4+CD25+ regulatory T cells are involved in maintaining tolerance and regulating the intensity of an IgE response but are not critical in preventing sensitization.

Allergic immune responses are attributed to the generation of Th2 cells that produce IL-4, -5, and -13 and generate IgE and eosinophilic responses. Murine models demonstrate that dendritic cells direct T cells toward a Th2 profile at the time of antigen presentation in the gut (33, 34). In order to explore the relative role of a Th2- or Th1-biased immune response in food allergy, T urcanu et al. (35) expanded human peanut-specific T cells from the peripheral blood with peanut antigen in vitro and then stimulated cells with phorbol 12-myristate 13-acetate (PMA) and ionomycin to maximize cytokine secretion. Expanded T cells from nine subjects with peanut allergy were interpreted to be Th2-biased with IFN-γ/IL-4 and IFN-γ/IL-13 ratios of 1:1 and 30:1, respectively, compared to ratios of 40:1 and 80:1 in the nonpeanut-allergic controls. However, Thottingal et al. (36) measured peanut-allergen-driven cytokine responses in short-term primary cultures of PBMCs in adults with peanut allergy and peanut-tolerant adults sensitized or not sensitized to peanut. Individuals with positive skin tests had more frequent or intense IL-5 and IL-13 responses than those without, whether or not they had clinically symptomatic peanut allergy. Surprisingly, the three groups were
Maillard reaction: glycosylation of amino groups to form stable advanced glycation end-products

not distinguishable by IFN-γ responses, which were absent, indicating that a “protective” Th1 bias does not explain the distinction in clinical outcomes, whereas a spectrum of Th2 responses may.

Homing and Localization of the Food Allergic Response

T cell homing to target organs may explain why food-allergic diseases such as food-associated atopic dermatitis or gastrointestinal disorders may occur despite a potential for systemic inflammation. For example, food-responsive T cells that bear the skin-homing receptor CLA (cutaneous lymphocyte antigen) are upregulated only in persons with food-responsive atopic dermatitis (37–39). Regarding isolated gastrointestinal allergies, the gut T cell homing molecule α-4, β-7 integrin is key in humans (40) and in murine models of food allergy (41, 42).

The immunopathophysiology of additional gastrointestinal food-allergy disorders is being elucidated. TNF-α from PBMCs cultured in vitro with triggering food proteins has been detected in infants with food-protein-induced enterocolitis syndrome (Table 1) (43). Chung et al. (44) found increased staining for TNF-α and decreased staining for the regulatory cytokine receptor TGF-β1 in duodenal biopsies of affected infants. Although more work is needed to elucidate the immunologic basis of this disorder, a deficit in TGF-β1 response and excessive TNF-α response may be important factors.

Eosinophilic esophagitis is an emerging disease characterized histologically by eosinophilic infiltration of the esophageal mucosa to a higher degree than observed with reflux (e.g., >15 cells/hpf) (45). In >90% of patients, eosinophilia resolves when all food allergens are eliminated from the diet (46). In studies utilizing gene microarray analysis of esophageal tissue, the mRNA encoding eotaxin-3 was the most highly upregulated transcript in eosinophilic esophagitis tissue compared to healthy control esophagus; esophageal eotaxin-3 mRNA and protein levels correlated with tissue eosinophilia; and a single-nucleotide polymorphism in the eotaxin-3 gene was associated with disease susceptibility (47). These studies indicate an allergen-specific relationship to food proteins and a crucial role for specific inflammatory pathways. Using biopsy specimens from patients with eosinophilic esophagitis and transient transfection experiments in esophageal cell lines, Blanchard et al. (48) determined that IL-13 plays a key role in the pathophysiology of the disorder and is a potential therapeutic target.

The Role of Food Proteins

Reactions to egg, milk, peanut, tree nuts, fish, shellfish, wheat, and soy account for most significant food allergies, although virtually any food may trigger an allergic response (1). Relatively few protein families account for most of the allergic reactions (49). Jenkins et al. (50) compared animal food allergens and human homologs by considering protein families, sequence analysis, and evolutionary relationships and noted that sequence identities to human homologs above 62% typically excluded a protein from being allergenic to humans. Features common to “major” food allergens are that they are water-soluble glycoproteins, are 10 to 70 kD in size, and are relatively stable to heat, acid, and proteases.

However, other attributes may also affect allergenicity. Although peanut consumption is nearly equivalent in China and the United States, those on western diets have higher rates of peanut allergy (51). In the United States peanuts are roasted, whereas in China they are typically boiled or fried. The high heat of roasting (180°C) peanuts leads to a Maillard reaction that appears to increase stability and allergenicity and is hypothesized to explain the discrepant allergy rates (52, 53). Additional characteristics of the food may be relevant. Shreffler et al. (54) showed that glycosylated Ara h 1, a major peanut allergen, but not the deglycosylated form, acted as a Th2 adjuvant by activating dendritic cells to drive the maturation of Th2
cells. Additionally, Ara h 1 acts as a ligand for DC-SIGN, which also has been shown to interact with schistosome glycoproteins and induce Th2 responses (55). Further exploration of specific molecular characteristics of food allergens and their interaction with the immune system is needed. For example, studies identifying the IgE-binding epitopes of allergens can determine potential sites for mutagenesis in the creation of novel vaccines (56).

**THERAPEUTIC APPROACHES**

Currently, the cornerstone of food allergy management is to vigilantly avoid trigger foods and maintain readiness to treat allergic reactions, for example with self-injectable epinephrine. Unfortunately, this approach is nutritionally and socially limiting, and reactions, including fatalities, nonetheless occur (12). Deficiencies in food allergy treatment contrast with the success of treatment of allergic diseases such as asthma, allergic rhinitis, and insect-venom anaphylaxis, for which anti-inflammatory therapies (such as topical steroids) and immunotherapies (such as subcutaneous immunotherapy with environmental or insect-venom allergens) are highly effective (57).

Subcutaneously delivered allergen immunotherapy results in allergen-specific immune deviation. Over time, IgE responses become blunted and allergen-specific IgG increases, reflecting a deviation from a Th2- to a Th1-dominant immune response. The possibility that subcutaneous immunotherapy could improve food allergy has been demonstrated in studies where birch pollen immunotherapy resulted in improvement in oral allergy syndrome (58). However, early attempts at immunotherapy utilizing injections with native peanut allergen resulted in modest clinical improvement but significant adverse effects, including recurrent anaphylaxis (59). Immunotherapeutic approaches now under study attempt to avoid serious adverse effects by changing the route of administration of native proteins, or by modifying (engineering) the treatment proteins. Additionally, a number of immunomodulatory treatments that are not antigen-specific are under study (Table 2).

**Allergen-Specific Therapies**

A number of therapies that are directed toward the treatment of specific food allergies are in clinical or preclinical trials. These approaches generally aim to alter the allergic response to the causal protein(s) without triggering an adverse immune response to the therapy itself. The benefit of these approaches is that, if effective, the food can be consumed in normal quantities with no symptoms. A limitation of these approaches is that they must be tailored specifically for each targeted food allergen. Treatment of peanut allergy has been a primary goal.

**Sublingual and oral immunotherapy with standard food allergens.** Gradual exposure of the allergic individual to native food proteins through the oral route could theoretically avoid acute allergic reactions that would otherwise be triggered by larger exposures, while simultaneously allowing for immune processing and immune deviation associated with oral tolerance. Sublingual immunotherapy (SLIT) with environmental allergens, which is being increasingly evaluated for treatment of respiratory allergy, induces regulatory T cells early in treatment and results in immune deviation toward Th1 responses later in therapy (60).

There are few studies of SLIT with foods. A randomized double-blind placebo-controlled trial evaluated the treatment of hazelnut allergy in adults with allergy confirmed by oral food challenge (n = 23, 12 on treatment) (61). Systemic reactions were observed in 0.2% of the total doses administered, only during the dose-escalation phase. After five months of treatment, the mean threshold dose of ingested hazelnut resulting in allergic symptoms in adults with allergy confirmed by oral food challenge increased from 2.3 g to 11.6 g (p = 0.02) in the active group, compared to an increase from 3.5 g to 4.1 g (not significant) in the placebo group. Levels of serum hazelnut-specific IgG4 antibody and total serum IL-10 increased only in the active group, but there were no differences in hazelnut-specific IgE antibody levels. A small
<table>
<thead>
<tr>
<th>Therapy</th>
<th>Immune strategy</th>
<th>Benefits</th>
<th>Limitations</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard subcutaneous immunotherapy (native allergens)</td>
<td>Antigen presentation in nonmucosal site results in Th1 skewing</td>
<td>Proven efficacy in venom and respiratory allergy, some studies show benefit for oral allergy syndrome</td>
<td>Pilot studies reveal anaphylaxis as side effect</td>
<td>No active development</td>
</tr>
<tr>
<td>Sublingual/oral immunotherapy</td>
<td>Antigen presentation to mucosal site provides “desensitization” and may induce “tolerance”</td>
<td>Natural foods, convenience, lower risk than injection immunotherapy</td>
<td>Can induce allergic reactions, may not induce tolerance</td>
<td>Numerous active protocols including sorely needed randomized controlled trials</td>
</tr>
<tr>
<td>Modified protein vaccine</td>
<td>Avoid activation of IgE by mutation of binding sites but maintain T cell responses</td>
<td>A safer form of immunotherapy compared to injection of native protein</td>
<td>Tedious production of relevant proteins. But use of E. coli as a delivery agent rectally bypasses some limitations</td>
<td>Effective in murine model, human studies planned</td>
</tr>
<tr>
<td>Peptide vaccine (overlapping peptides)</td>
<td>Avoid activation of IgE by lack of peptides large enough to cross-link IgE but maintain T cell responses</td>
<td>No requirement for IgE epitope mapping/mutation</td>
<td>Hard to characterize large number of peptides</td>
<td>Murine models only</td>
</tr>
<tr>
<td>Conjugation of immune stimulatory sequences to allergen</td>
<td>Enhance Th2 response by activating innate immune receptors, possibly hinder IgE binding</td>
<td>Increased efficacy, possibly improved safety</td>
<td>Conjugation may alter relevant protein</td>
<td>Some promise shown in human studies using environmental allergens</td>
</tr>
<tr>
<td>Plasmid DNA encoded vaccines</td>
<td>Endogenous production of allergen may result in tolerance</td>
<td>Possible one-dose treatment</td>
<td>Murine models reveal strain-specific response</td>
<td>No active development</td>
</tr>
<tr>
<td>Anti-IgE antibodies</td>
<td>Bind and inactivate IgE while it is not bound to high-affinity IgE receptors</td>
<td>Potentially useful for any food allergen. Some response in eosinophilic gastroenteropathy (pilot study)</td>
<td>Preliminary study did not show uniform protection, some improved threshold. Not a cure</td>
<td>Studies on hold, various study designs being considered</td>
</tr>
<tr>
<td>Chinese herbal medicine</td>
<td>Mechanism unknown, not simply immune suppression, not steroid effect</td>
<td>“Natural” treatment. Not food-specific</td>
<td>Currently unknown</td>
<td>Preclinical studies promising, human safety studies under way</td>
</tr>
<tr>
<td>Cytokine/anticytokine</td>
<td>Interrupt inflammatory signals</td>
<td>May allow directed interruption of inflammatory processes or prevention of sensitization</td>
<td>May interrupt beneficial protective immune responses</td>
<td>Efficacy studies under way for anti-IL-5 in eosinophilic esophagitis</td>
</tr>
</tbody>
</table>
There are a growing number of studies of oral immunotherapy (OIT), in which a food allergen is ingested in gradually increasing amounts over months, but there have been no randomized controlled trials. The more recent and larger studies (63–66) target treatment primarily for egg and milk allergy in children. The success rates vary, probably because of patient selection, dosing regimens, and definition of response, but are generally in the range of 70%–80%. Failures appear more common in those with higher allergen-specific IgE levels at the outset (64, 66), although a recently published protocol was at least partially successful in 90% of 30 children with severe milk allergy and highly elevated milk-specific IgE (67). The procedures are not without side effects, including systemic allergic reactions, and it appears that some subjects experience reactions to dosing even while on stable doses when they exercise or experience infections (66).

OIT is presumed to restore or induce a tolerant state. However, a distinction must be recognized between desensitization, where the allergen is ingested without symptoms during treatment but has to be ingested daily, and tolerance, where the food may be ingested without allergy symptoms despite periods of abstinence. Studies to date indicate that OIT induces desensitization, but some evidence suggests that it does not induce tolerance (68). Staden et al. (66) randomized children to egg or milk OIT (n = 25) or observation during dietary elimination (n = 20); after oral food challenges at ~21 months on therapy, the treatment group discontinued daily therapy for two months and were rechallenged. Although 64% of the treatment group had had a good or at least partial response to OIT while on treatment, food challenges performed two months post-treatment revealed that only 36% continued to have true tolerance, a percentage that exactly matched tolerance achieved in untreated controls. More studies are clearly required to assess safety, efficacy, and mechanisms of OIT.

**Engineered proteins.** One approach to avoid activating mast cells during specific allergen immunotherapy is to identify IgE binding sites on proteins and mutate the sites to ablate IgE binding, while preserving the protein’s ability to stimulate T cells. This strategy has been successfully utilized to generate hypoallergenic mutants of peanut (69, 70), fish (71), and apple allergens (72). An example is the modification of peanut proteins delivered for therapy in *E. coli* by the rectal route. A bacterial vector was selected to enhance Th1 and regulatory responses, and the rectal route was selected to avoid adverse effects of injecting whole bacteria.

In initial studies (73), peanut-sensitized mice were treated with heat-killed *E. coli* containing modified Ara h 1–3, vector alone, or placebo. Mice treated with the rectal vaccine had the lowest symptom scores, least decrease in body temperature, and least rise in plasma histamine following peanut challenge. Splenocytes from the treated mice cultured in vitro in the presence of peanut protein produced significantly less IL-4, IL-5, and IL-13, and more IFN-γ and TGF-β, than placebo-treated mice. Clinical studies are planned.

**Peptide immunotherapy.** Another strategy is to create a vaccine composed of numerous small peptides that span the sequence of native allergenic proteins with sequential overlap (74). This strategy presents T cell epitopes but avoids cross-linking of IgE. Preliminary studies (74a) in a murine model show promise, but the practical problem of validating the stability and uniformity of a complex peptide vaccine has stalled development.

**Plasmid DNA.** A third interesting approach is to utilize bacterial plasmid DNA encoding an allergen for immunotherapy. The plasmid DNA strategy builds on the hypothesis that endogenously produced antigen would not stimulate an allergic immune response. This strategy was successful in preventing peanut allergy in a murine model when a chitosan-embedded plasmid that contained DNA encoding the peanut allergen Ara h 2 was administered orally to
Pathogen-associated molecular patterns: microbial products such as lipopolysaccharides that are recognized by receptors of the innate immune system, such as toll-like receptors.

AKR/J mice (75). However, a subsequent study using intramuscular immunization in sensitized mice revealed protection for AKR/J mice but worse outcomes for treated C3H/HeJ mice. Such strain-dependent outcomes suggest variable outcomes in humans (76).

Modifications using immune stimulatory sequences. Another vaccine strategy is to attach to allergens determinants that activate nonallergic immune responses. Pathogen-associated molecular patterns, such as unmethylated cytosine and guanine motifs (CpG motifs), are examples of such determinants. They act as immune stimulatory sequences (ISSs) that simultaneously provide steric hindrance to reduce IgE binding and to enhance Th1 responses. In one study, C3H/HeJ mice were immunized intranasally with ISS-linked Ara h 2 (ISS-Ara h 2), or ISS-linked Amb a1, the major ragweed allergen, as a control. Four weeks following initial immunization, mice were sensitized with peanut intragastrically by gavage and then challenged with Ara h 2 five weeks later (76a). ISS-Ara h 2–treated mice had reduced symptoms and lower plasma histamine following oral challenge with Ara h 2 compared to controls. In a similar study, Nguyen et al. (77) found that intradermal immunization with a mixture of oligodeoxynucleotide (ODN) ISS and β-galactosidase, but not with either ISS ODN or β-galactosidase alone, provided significant protection against fatal anaphylactic shock induced by intraperitoneal β-galactosidase sensitization and challenge. Taken together, these data suggest that antigen- ISS ODN immunization may prevent food allergy, but whether it will ameliorate established food allergy remains unexplored.

Therapies That Are Not Allergen-Specific

In contrast to the allergen-specific therapies, several strategies have been explored to downregulate allergic immune responses. Some of these approaches (e.g., anti-IgE) may only alter the threshold of allergic reactivity; others show promise for more permanent cures of food allergy (e.g., Chinese herbal medicine).

Anti-IgE. Because IgE antibodies are centrally involved in acute allergic reactions, their neutralization would be expected to reduce or eliminate food-induced anaphylaxis. A double-blind placebo-controlled dose-ranging clinical trial of a humanized monoclonal anti-IgE, TNX-901, was carried out in 84 volunteers with peanut allergy (78). The 450-mg dose of TNX-901 significantly increased the threshold of reactivity to peanut by oral food challenge from 178 mg (≈1/2 peanut) to 2.8 g (≈9 peanuts) ($p < 0.001$). However, the therapeutic response was not uniform; ≈25% of subjects experienced no change in their threshold of reactivity and another 25% tolerated at least 10 g (>20 peanuts). The reason for this disparity remains unclear, since no biomarkers studied predicted the outcome. Because another anti-IgE product was under development for asthma, the development of TNX-901 was discontinued, but its ability to provide a level of protection is encouraging. A controlled trial with a different anti-IgE antibody preparation [omalizumab (Xolair®, Genentech] was initiated to evaluate its effectiveness for treatment of peanut allergy, but it was discontinued before significant results could be obtained because of safety issues with the protocol (79). Additional studies of anti-IgE therapy are needed to confirm its level of protection and utility.

Omalizumab was also piloted as a treatment for eosinophilic gastroenteritis in nine subjects treated biweekly for 16 weeks (80). Symptom scores decreased (70%, $p < 0.005$); eosinophil counts tended to decrease in the duodenum (59%, $p = 0.07$) and gastric antrum (69%, $p = 0.1$) but not in the esophagus (increased 25%, $p = 0.5$). These modest preliminary results indicate that IgE-associated mechanisms may contribute to eosinophilic gastroenteropathies.

Chinese herbal medicine. Li and colleagues have developed a nine-herb preparation, designated FAHF-2, which in one study completely blocked anaphylactic symptoms in a murine...
model of peanut allergy (81). Following the daily administration of FAHF-2 or placebo to peanut-sensitized mice for six weeks, mice were challenged monthly to determine the extent of protection. Anaphylactic symptoms occurred with each peanut challenge in placebo-treated mice, whereas the FAHF-2-treated mice were fully protected until the sixth month, when retreatment afforded renewed protection. Compared to controls, the FAHF-2-treated mice had significantly lower plasma levels of peanut-specific IgE following therapy, and splenocytes cultured in vitro with peanut protein produced less IL-4, IL-5, and IL-13 and more IFN-γ. Phase I safety studies in humans have been initiated (82).

Cytokines and anticytokines. Froussard (83) used Lactobacillus engineered to deliver IL-10, a suppressive regulatory cytokine, to diminish sensitization and reactivity in a murine model of milk allergy. Ando et al. (84) tested TGF-β, a protein thought to induce tolerance when ingested orally (e.g., in breast milk). BALB/c mice treated orally with ovalbumin and TGF-β showed reduction of ovalbumin-specific IgE and IgG1 antibodies, T cell reactivity, and immediate-type skin reactions compared with mice treated orally with ovalbumin alone. Stein et al. (85) undertook an open-label phase I/II study of a humanized monoclonal IgG antibody against IL-5 in four adults with eosinophilic esophagitis. Following three monthly infusions, mean esophageal eosinophil counts fell from 46 to 6 per high-power field ($p < 0.001$), and clinical symptoms and quality of life improved.

Additional Strategies

Other therapeutic strategies using immunostimulation of Th1 and regulatory pathways with various agents while simultaneously presenting allergens show promise. Heat-killed Listeria monocytogenes (HKL) stimulate IFN-γ production by CD4 T lymphocytes. This approach was studied in a pilot protocol where food-allergic dogs were vaccinated once subcutaneously with peanut, milk, or wheat with HKL emulsified in incomplete Freund’s adjuvant. Following vaccination, oral challenges elicited only minor or no symptoms, and skin-test end-point titrations showed marked reductions (86). A similar protective effect was demonstrated in a murine model of peanut-induced anaphylaxis (87). In a murine model, Zhu et al. (88) used a synthetic agonist of toll-like receptor 9 (immune modulatory oligonucleotide) in prevention and treatment protocols of orally induced peanut allergy and found reduced Th2 and increased Th1 responses, reduced symptom scores, and reduced gastrointestinal inflammation in the treated animals. Combination strategies may be possible, such as the use of anti-IgE antibodies to quell allergic reactions to immunotherapy whether performed orally or by injection. Studies on immunotherapy of environmental allergies may provide additional insights that can be applied to foods. For example, Creticos et al. (89) recently showed efficacy and safety of a vaccine composed of ragweed allergen linked to a toll-like receptor 9 agonist, a phosphorothioate oligodeoxyribonucleotide immunostimulatory sequence of DNA. Finally, in murine models (90), by engineering proteins that target the dominant-negative signaling receptor FcγRIIb expressed on proallergic cells, it may be possible to repress IgE responses or enhance the safety of immunotherapy.

CONCLUSION

We are gaining an increasing appreciation of the immune mechanisms underlying food-allergic responses and of how tolerance may be abrogated or bypassed to result in food allergy. Although there is a genetic disposition toward atopy and food allergy, the explanations for the rise in prevalence of food allergy must lie in environmental factors that presumably include global influences on immune responses. Subtleties such as dose and timing of food exposures and food-processing methods that alter allergenic potential may influence disease expression and outcomes. Though only partially
understood, these factors present opportunities to explore better targets for disease prevention and treatment. The numerous avenues for intervention that are now being explored in pre-clinical and clinical studies (Table 2) offer hope for better treatments in the near future.

**SUMMARY POINTS**

1. Food allergy is an adverse immune response to food proteins.
2. Food allergy is an important problem because it is increasing in prevalence, lacks current curative therapies, and can be life-threatening.
3. Characteristic clinical features of food allergy derive from underlying immune responses that are IgE-antibody-associated (acute allergic reactions, anaphylaxis), cell-mediated (chronic gastrointestinal disorders), or both.
4. Food allergy represents a breach in normal oral tolerance despite ingestion of the inciting protein, or it may result from a sensitizing exposure through the skin or respiratory tract that bypasses oral tolerance.
5. Normal oral tolerance requires an active gut mucosa that provides a physical and immunological barrier. At this barrier, antigen-presenting cells and T regulatory cells are active, and controlled inflammation occurs in response to foreign food proteins and commensal gastrointestinal flora without evoking adverse immune consequences.
6. Certain characteristics of proteins and circumstances of exposure increase the potential of a food to be allergenic. Properties of commonly allergenic proteins include stability to heat and digestion, stable particulate structures (that may occur from heating), and glycosylation that may mimic parasite molecular patterns that induce allergic immune responses. The likelihood of developing an allergy is heightened by increased gastric pH or young age and is sensitive to the frequency, timing, route, and dose of exposure.
7. Allergic responses typically result from immune deviation toward a Th2 response, where IL-4, IL-5 and IL-13 induce IgE antibodies and eosinophilic inflammation resulting in disease.
8. Food-allergen-specific immunotherapeutic approaches are devised to present allergen in a context that stimulates a Th1 immune response (e.g., IFN-γ) or generates suppressive (regulatory) T cells, and results in downregulation of Th2 responses, without triggering an allergic reaction.
9. Therapies that are not allergen-specific target immune mediators such as IgE and cytokines to modulate or interrupt allergic responses.

**FUTURE ISSUES**

1. Identify dietary, genetic, and environmental influences that induce or increase the risk of allergic responses to foods.
2. Determine the relative influences of exposure to food proteins on allergy outcomes regarding maternal ingestion of allergens during pregnancy and lactation and the timing of introduction of foods during weaning.
3. Understand the interplay of antigen processing and immune regulation with regard to outcomes of allergy or tolerance to specific proteins.

4. Evaluate the safety and efficacy of sublingual and oral immunotherapy for food allergy. Determine whether this type of therapy can induce oral tolerance or merely desensitization.

5. Determine the mechanisms of effectiveness of Chinese herbal therapies for food allergy.

6. Carry out clinical studies to determine the efficacy of various treatment strategies to create safe and effective curative therapies.

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81. This Chinese herbal medicine was completely effective in treating peanut allergy in a murine model of peanut anaphylaxis.
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