

Novel approaches for the treatment of food allergy

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At present there is no effective therapy for IgE-mediated food allergy, and patients must rely upon food-allergen avoidance. Unfortunately, the accidental ingestion of allergen-containing foods leading to potentially severe reactions is common. Definitive therapies for food allergy are desperately needed. Although rush immunotherapy has been reported to induce tolerance in some patients, the rate of maintenance of tolerance was low, and there was an undesirably high incidence of adverse reactions. New knowledge of the immunological mechanisms underlying allergic disease has expanded the potential therapeutic options for food allergy. The establishment of animal models of food hypersensitivity, which include sensitization by the oral route and anaphylaxis upon oral challenge, have facilitated the investigation of therapies for food allergy. Novel approaches under investigation include the reduction of IgE by the infusion of anti-IgE antibodies, vaccination with plasmid DNA, the use of anti-allergy immunostimulatory sequences, cytokines and bacterial agents, immunotherapy with mutated proteins and peptides, and complementary medicine such as Chinese herbs. *Curr Opin Allergy Clin Immunol* 2:273–278. © 2002 Lippincott Williams & Wilkins.

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Abbreviations

FAHF-1	food allergy herbal formula-1
HKE	heat-killed <i>Escherichia coli</i>
HKL	heat-killed <i>Listeria</i>
ISS	immunostimulatory sequence
TGF-β	transforming growth factor beta
Th	T helper cell

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Introduction

Food allergy currently affects approximately 2% of the US population, appears to be increasing, and is now the most frequent cause of anaphylactic reactions treated in emergency departments in western countries [1–4]. At present there is no effective therapy for IgE-mediated food allergy.

The generation of allergen-specific IgE antibodies that bind to high affinity receptors (Fc ϵ RI) on the surface of mast cells and basophils is central to the immunopathology of food allergy and other atopic disorders. In patients with food hypersensitivity, re-exposure to the relevant foods triggers the degranulation of mast cells/basophils resulting in the release of histamine and other mediators, which provoke symptoms of anaphylaxis. As in other allergies, food allergy is mediated by a T helper cell (Th) type 2 immunological response. The ‘hygiene hypothesis’ postulates that reduced pathogen exposure during infancy in modern western environments influences the maturation of the infant immune system from a Th2 to a Th1 type of immune response; the loss of this influence has presumably promoted the recent epidemic of extrinsic asthma and other allergic diseases [5]. This knowledge has led to new immunotherapeutic strategies for the treatment of food allergy aimed at re-establishing the balance between Th1 and Th2 responses or blocking the IgE–mast cell interaction. In addition, Chinese herbal medicines that exhibit anti-allergy properties are beginning to be investigated as an alternative treatment for food allergy. Because of concerns over anaphylactic reactions in patients, animal models are being developed to test these potential therapies. Li and colleagues [6,7], for example, recently developed murine models of IgE-mediated cows’ milk hypersensitivity and peanut anaphylaxis using oral sensitization and challenge, in which the symptoms and T and B cell responses to food allergens resembled those seen in food-allergic patients. These animal models are useful tools for investigating novel approaches to food allergy therapy. This article presents an update on progress in the development of therapeutic approaches aimed at the desensitization of IgE-mediated food allergy.

DNA-based therapies

Two types of DNA based immunotherapies – plasmid DNA-based and oligodeoxynucleotide-based immunotherapies – have been investigated in animal models of food allergy.

Plasmid DNA-based immunotherapy

Plasmid DNA-based immunotherapy (DNA vaccine) is a novel method of generating immune responses by immunizing with bacterial plasmid DNA (pDNA), encoding specific antigens. DNA vaccination can induce prolonged humoral and cellular immune responses, and is of particular interest in the treatment of allergy because it induces a Th1 response [8], attributable to immunostimulatory sequences (ISSs) consisting of unmethylated cytosine and guanine motifs (CpG motifs) in the pDNA backbone.

Although almost any food protein can cause hypersensitivity reactions, most adverse reactions are caused by milk, egg, peanut, soybeans, wheat, fish, shellfish and tree nuts [9•]. Because peanut accounts for most life-threatening food allergic reactions [1], several recent investigations have focused on peanut allergen gene therapy. Burks and colleagues [10,11] identified three peanut allergens: Ara h1 (63 500 M_r) and Ara h2 (17 000 M_r) are the two major peanut allergens, recognized by 95% of peanut-allergic patients; Ara h3 (14 000 M_r), the minor peanut allergen, is recognized by 40% of peanut-allergic patients [12]. The characterization and cloning of peanut allergens has made experimental pDNA-based peanut allergen gene therapy and other immunotherapies possible. An early study [13,14] found that the intramuscular immunization of naïve AKR/J (H-2^K) and C3H/HeN (H-2^K) mice with pDNA encoding Ara h2 before to intraperitoneal peanut sensitization had some protective effect in AKR/J mice, but induced anaphylactic reactions in C3H/HeN mice upon peanut challenge. Roy *et al.* [15] demonstrated that the oral administration of chitosan embedded pAra h2 also had a protective effect in AKR mice. The success in one strain but failure in another may reflect a potential for problems if this therapy were to be applied to humans. Although prophylactic protocols have provided some basis for the use of immunoregulatory modulators in food-allergic disorders, in order to be clinically relevant, therapeutic strategies must ameliorate an established allergy. There are currently no published reports of successful pDNA-based immunotherapy of 'established' peanut or other food allergy. We tested a possible therapeutic effect of pDNA-based therapy in peanut-sensitized mice using the previously reported prophylactic pAra h2 immunization regime, and found no reduction in IgE levels (Li *et al.*, unpublished data). These results suggest that the current form of pDNA-based immunotherapy may not be effective in reversing IgE-mediated hypersensitivity, and that additional methods that improve the efficacy of peanut allergen DNA immunization need to be developed. Modalities to improve pDNA-based therapy include the generation of new gene constructs containing additional CpG motifs, the addition of modulatory cytokine genes, which

enhance the Th1 response, encoding the plasmids for the synthesis of modified food allergens (that reduce the risk of inducing anaphylactic reactions), and optimizing the gene delivery pathway and DNA doses.

Immunostimulatory oligodeoxynucleotide-based immunotherapy

There is growing interest in the use of synthetic immunostimulatory oligodeoxynucleotides containing unmethylated CpG motifs (ISS) for the prevention or treatment of allergic disorders such as allergic asthma [16,17]. It has been shown that the administration of ISS-conjugated antigen was more effective than a mixture of antigen and ISS in the suppression of allergic airway responses [18] probably because of the enhanced dendritic cell uptake of ISS allergen [19]. In collaboration with Dynavax Inc. (Berkley, CA, USA) we have begun investigating the use of ISS-conjugated-Ara h2 in our mouse model of peanut-induced anaphylaxis [20]. C3H/HeJ mice were immunized intradermally with ISS-linked Ara h2, or ISS-linked Amb a1 (ISS-Amb a1) as a control. Four weeks after the initial immunization, mice were intragastrically sensitized with peanut and then challenged with Ara h2 5 weeks later. ISS-linked Ara h2-treated mice did not develop obvious symptoms after oral challenge with Ara h2, whereas ISS-linked Amb a1-treated mice did. Plasma histamine levels were also significantly lower in the ISS-linked Ara h2-treated group than those in the ISS-linked Amb a1-treated group. These findings suggest that ISS-linked Ara h2 immunization had some preventive effect on the peanut-induced allergic response in an antigen-specific manner. Nguyen *et al.* [21•] recently found that intradermal immunization with a mixture of immunostimulatory oligodeoxynucleotides and β -gal, but not with either immunostimulatory oligodeoxynucleotides or β -gal alone, provided significant protection against fatal anaphylactic shock induced by β -gal intraperitoneal sensitization and challenge. This effect was comparable to immunization with pACB-LacZ, the pDNA encoding β -gal. The study also found that the protective effect of ISS plus β -gal immunization was associated with an increase in Th1 (IgG2a/IFN- γ) and a reduction in Th2 (IgE/IL-4, IL-5) patterns. Taken together, these data suggest that antigen-immunostimulatory oligodeoxynucleotide immunization may have a prophylactic effect against allergy. However, an ability to desensitize established food allergy remains to be determined.

Mutated protein immunotherapy and peptide immunotherapy

Immunological tolerance is believed to be induced by redirecting the T-cell immune response from a Th2 to a Th1 type response [22,23]. A technique that generates engineered major allergenic food proteins, through alteration of the primary amino acid sequences of IgE-

binding allergenic epitopes of the major allergens present in foods, has recently been developed. These modified proteins exhibit properties favorable for allergen immunotherapy in in-vitro assays [24–26]. Stanley *et al.* [26], for example, showed that the engineered recombinant proteins Ara h1 and Ara h2 bind virtually no IgE antibodies from peanut-allergic patients, but promote comparable T-cell proliferation as the native peanut proteins. In collaboration with Drs Burks and Bannon (University of Arkansas, USA), we recently tested the in-vivo efficacy of modified peanut allergen on our murine model of peanut anaphylaxis. Mice were sensitized with whole peanut and then desensitized by the intranasal administration of modified Ara h2 (three doses a week for 4 weeks). Desensitization with the modified Ara h2 protein resulted in reduced amounts of Ara h2-specific IgE, and significantly lowered anaphylaxis scores when compared with controls (Li *et al.*, unpublished data).

The use of overlapping peptides is also being investigated as an alternative approach. Vaccines consisting of small peptides (10–20-mers) that represent the entire length of a specific food protein are generated. For example, with Ara h2, we have generated 30 20-mers that overlap by 15 amino acids. In this way, antigen-presenting cells (APC) are provided with all the possible T-cell binding epitopes, but mast cells are not activated because these peptides are unable to cross-link two IgE molecules. Preliminary studies in our anaphylactic mouse model showed that desensitization with a 20-mer Ara h2 peptide mixture (three doses a week for 4 weeks) reduced Ara h2-specific IgE, significantly lowered plasma histamine levels and anaphylaxis scores, and increased IFN- γ production by spleen cells when compared with controls (Li *et al.*, unpublished data).

Whereas both mutated protein immunotherapy and peptide immunotherapy seem promising, peptide immunotherapy has several advantages over mutated protein immunotherapy: IgE binding sites for each food protein do not need to be mapped, allergen-specific DNA does not need to be mutated, and engineered recombinant proteins are not required. Using this approach, vaccines against any food in which the responsible allergenic proteins are known can be formulated.

Bacterial-based immunotherapy

Two types of bacteria-based immunotherapy therapies including probiotics and heat-killed bacteria as adjuvant have been investigated for food allergy.

Probiotics

Probiotics are live bacteria or components of microbial cells, which have beneficial effects on the health and

well-being of the host [27]. According to the conventional probiotic concept formulated nearly 100 years ago, beneficial bacteria are regarded as food supplements, which act as non-specific balancers of the gut microflora and potentiators of immunity. The major consumption of probiotics is in the form of dairy-based foods containing lactobacilli and bifidobacteria. Probiotics have had little impact on food allergy treatment because of the limited amount of scientific validation for their efficacy. However, Majamaa and Isolauri [27] for the first time in a randomized, double-blind controlled clinical study found that *Lactobacillus rhamnosus* GG alleviated symptoms of atopic dermatitis associated with milk allergy in children. Symptomatic improvement was associated with the promotion of IgA and the suppression of TNF- α synthesis [27]. More recently, in another double-blind, randomized, placebo-controlled trial, lactobacillus GG was given prenatally to mothers who had at least one first-degree relative (or partner) with atopic eczema, allergic rhinitis, or asthma, as well as postnatally for 6 months to their infants. The frequency of atopic eczema in the probiotic-treated group was half that of the placebo group, suggesting that lactobacillus GG had some effect in the prevention of early atopic disease in children at high risk [28•]. The mechanisms underlying the reported effects of lactobacillus GG on atopic dermatitis are not clear. However, it appears that lactobacillus GG has anti-allergic inflammatory properties evidenced by the recent report that lactobacillus rhamnosus GG increased IL-10 in the sera of children with atopic dermatitis and milk allergy [29], and that *L. rhamnosus* GG-degraded casein suppressed T-cell activation accompanied by a reduction of both IL-4 and IFN- γ [30].

Heat-killed *Listeria*

Heat-killed *Listeria* (HKL) is a strong adjuvant, which has been shown to induce IFN- γ production and to reverse established allergic airway hyperreactivity in mice [31,32]. Recently, Frick *et al.* [33] found that a single subcutaneous treatment with a mixture of HKL and milk and wheat emulsified in incomplete Freund's adjuvant significantly reduced immediate skin test reactions and eliminated oral challenge-induced symptoms. In collaboration with Drs Burks and Bannon at the University of Arkansas, and Dr Bottomly at Yale University School of Medicine, we are investigating the efficacy of desensitizing peanut hypersensitivity with a mixture of HKL and modified Ara h1, 2 and 3 proteins, as well as heat-killed *Escherichia coli* (HKE)-producing modified Ara h1, 2 and 3 proteins in our murine model of peanut hypersensitivity. In that study, desensitization was initiated 10 weeks after sensitization (once a week for 3 weeks). The preliminary results showed that both approaches could significantly reduce peanut-specific IgE production and inhibit peanut-induced anaphylactic

reactions (Li *et al.*, unpublished data). We also found that these two approaches require lower doses for desensitization than either modified peanut protein or peptide immunotherapies, suggesting that allergen immunotherapy with HKL or HKE as adjuvants may be useful approaches for food allergy treatment. However, the safety of HKL and HKE bacterial therapy remains to be determined.

Anti-immunoglobulin E therapy

Humanized, monoclonal anti-IgE antibodies, which bind to the third domain of the Fc region of IgE molecules, thereby preventing their binding to the Fc receptors Fc ϵ RI or Fc ϵ RII have recently been developed. A number of clinical trials [34–38] utilizing these antibodies have reported a symptomatic improvement in patients with allergic asthma and rhinitis when circulating levels of IgE antibodies are markedly reduced. In addition, it has been found that basophil histamine release and Fc ϵ RI expression are downregulated during anti-IgE therapy [39]. In conjunction with five other centers in the USA, we have initiated a double-blind placebo-controlled trial of Tanox's humanized, monoclonal anti-IgE antibody in the treatment of patients with peanut-induced anaphylaxis. To date, 84 patients have been enrolled in this double-blind trial. In that study, the quantity of peanut necessary to provoke an allergic reaction is established by double-blind placebo-controlled food challenge. Patients then receive four subcutaneous injections of anti-IgE or placebo at monthly intervals and then undergo a second peanut challenge. If successful, this form of therapy could provide food-allergic patients with relief from virtually any food allergen, although continued protection would depend on indefinite monthly injections.

Cytokine therapy

On the basis of their cytokine profiles, at least two functional subsets of CD4 T cells have been identified [40–42]. Th1 cells express IFN- γ , whereas Th2 cells produce IL-4, IL-5 and IL-13. Numerous studies have demonstrated that Th2 cytokines play a central role in the pathogenesis of allergic asthma. IL-4/IL-13 promotes B-cell switching to IgE production and mast cells activation. IL-5 has also been shown to have a potentially autocrine effect on mast cells, in addition to its recognized paracrine effects on eosinophils [43,44]. A Th2 skewed response in food allergy has also been demonstrated in patients and animal models [6,45]. Schade *et al.* [45], for example, recently showed that T-cell clones generated from infants with cows' milk allergy produced high levels of IL-4, IL-5 and IL-13, and low levels of IFN- γ , whereas infants without cows' milk allergy had high levels of IFN- γ and low levels of IL-4, IL-5, and IL-13. Interestingly, Th1 and Th2 cytokines promote the growth/differentiation of their subset and inhibit the growth/differentiation of the

opposing subsets [46]. The suppression of Th2 responses by directly administering Th1 cytokines (IL-12, IFN- γ), or neutralizing antibodies against Th2 cytokines (IL-4, IL-5 or IL-13), or their receptors may thus be interesting strategies for food allergy treatment. IL-12, a heterodimeric cytokine produced by antigen-presenting cells, promotes the differentiation of Th1 cells and IFN- γ production, and inhibits the differentiation of Th0 cells into IL-4-secreting Th2 cells, thereby suppressing IgE production [47–51]. Intraperitoneal IL-12 treatment of mice has been shown to inhibit antigen-induced eosinophilic inflammation, airway hyperresponsiveness, and IgE production [52–54], and to switch a Th2- to a Th1-type response in established *Leishmania major* infections [55]. Recently, Lee *et al.* [56•] used a mouse model of peanut anaphylaxis to evaluate the possible prophylactic and therapeutic effects of orally administered IL-12 on peanut allergy. The authors found that oral IL-12 administration initiated 3 weeks after sensitization as well as at sensitization attenuated the anaphylactic reactions triggered by peanut challenge of allergic mice. Symptom reduction was accompanied by a reduction in peanut specific-IgE, and a reversal or reduction of the IgG1:IgG2a ratio. Furthermore, oral IL-12 treatment increased the IFN- γ :IL-4 and IFN- γ :IL-5 ratios. These results suggest some potential for the use of IL-12, either alone or in combination with other immunomodulatory agents, as a treatment for peanut allergy. Further studies are required to evaluate the dose-related and long-term effects of IL-12 therapy on peanut hypersensitivity.

IFN- γ therapy for atopic dermatitis has had limited success [57]. However, Lee *et al.* [58] recently found that the clinical results of IFN- γ therapy for atopic dermatitis were enhanced by an elimination diet. The combination of IFN- γ therapy and an elimination diet also produced more clinical improvement than either the elimination diet or IFN- γ alone.

In contrast to asthma, there has been no research into the efficacy of anti-Th2 cytokine antibodies, such as anti-IL-4 or anti-IL-5 on food allergy. Transforming growth factor beta (TGF- β), which is classified as Th3 cytokine, is an important immunoregulatory cytokine in oral tolerance. It has been found that colostrum TGF- β concentrations were lower in samples from mothers of infants with IgE-mediated cows' milk allergy than in samples from mothers of infants with non-IgE-mediated cows' milk allergy [59]. Atopic dermatitis has also been associated with a low-producer TGF- β -1 cytokine genotype [60]. It would be of interest to test whether TGF- β administration could be of benefit in food allergy.

Alternative medicine

Traditional Chinese medicine, used in Asia for centuries, is attracting interest in Western countries as a source of

alternative or complementary therapies for a variety of diseases, because of its reputed effectiveness, low cost, and relative absence of side-effects. Previous studies including ours [61–63] provided scientific evidence to support the use of traditional Chinese medicine for allergic asthma. Recently, Li *et al.* [64**] investigated the effect of a traditional Chinese medicine herbal formula, food allergy herbal formula-1 (FAHF-1), for the treatment of peanut allergy in a well-characterized murine model of peanut hypersensitivity, and found that FAHF-1 markedly reduced mast cell degranulation and histamine release, and completely blocked peanut-induced anaphylactic symptoms. Peanut-specific serum IgE levels were significantly reduced by 2 weeks of treatment, at the time of challenge, and remained lower for 4 weeks after the discontinuation of treatment. FAHF-1 significantly reduced peanut-induced lymphocyte proliferation, and IL-4, IL-5 and IL-13, but not IFN- γ synthesis. No toxic effects on liver or kidney functions and no overall immune suppression was observed. Although animal models are not identical to human disease, the study suggested that FAHF-1 and other herbal formulas may be useful for the treatment of peanut allergy and other IgE-mediated food allergy. Although the mechanisms are not fully understood, it is possible that FAHF-1 may target many parts of the food allergic reaction cascade, such as suppressing antigen-specific B cells, Th2 cells and mast cell activation. It is also conceivable that FAHF-1 may reduce intestinal permeability, thereby reducing the amount of peanut allergens available to interact with mast cells. These features may prove to be particularly advantageous, even over other immunotherapies that only target antigen, Th2 cytokines, or IgE antibody. Research is underway to develop standardized herbal products for clinical study.

Conclusion

Although there is no effective and safe therapy for food allergy, many novel approaches are under investigation. Some of these approaches are undergoing clinical trials and may be available to allergists in the near future.

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