

Current developments in peanut allergy

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Purpose of review

Peanut allergy is among the most serious, life-threatening food sensitivities, and recent studies indicate increasing prevalence, particularly among children. Our objective is to highlight recent advances in the immunology and treatment of peanut allergy.

Recent findings

Peanut sensitization may be both a Th1- and Th2-driven process, and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) may play a role in regulating the response intensity. Preliminary work shows that the food matrix is important in the immune response to peanut and that purified peanut allergens may have little intrinsic stimulatory capacity. Studies characterizing peanut allergens have revealed Ara h 1 and Ara h 2 as the most potent allergens, but Ara h 3 may be more allergenic than previously thought. There appears to be a relationship between the diversity of IgE-binding patterns and the severity of clinical symptoms. Multiple novel approaches to treatment are being investigated, which include traditional Chinese medicine, various forms of modified immunotherapy and the use of adjuvants in modified immunotherapy.

Summary

By understanding the immunologic response to peanut and the roles of the major peanut allergens, it may be possible to predict those at risk for severe reactions, prevent peanut sensitization and effectively treat those already sensitized.

Keywords

allergen-specific immunotherapy, food allergy, mutated allergen, peanut hypersensitivity, peanut sensitization

Introduction

Approximately 0.5–1% of the population is affected by peanut allergy, and a recent study shows that prevalence in children is increasing [1]. It is estimated that as many as 150 deaths occur each year due to food allergy, and that a majority of these are due to peanut allergy [2]. Furthermore, since only 20% of patients lose their sensitivity, peanut allergy is usually lifelong. Those who do lose sensitivity can have recurrence that is associated with continued avoidance of the allergen [3]. Currently, the only available treatment is strict avoidance of all peanut products and timely treatment of accidental ingestions with antihistamines and self-injectable epinephrine. In recent years, much progress has been made in elucidating the immunologic response to peanut, characterizing specific peanut allergens, and in developing alternative therapies for peanut allergy.

Immunologic response to peanut

The investigation of treatment for peanut allergy has been targeted to individuals already sensitized to peanut. However, there may be opportunities to intervene therapeutically to prevent sensitization. Not much is known about the immunologic process of sensitization, but interest in this area is increasing. The development of murine models of sensitization, oral tolerance and peanut anaphylaxis has been vital in this regard [4–6].

A murine model was recently used to investigate early immune responses to crude peanut extract (CPE) and Ara h 1, 2, 3 and 6 during oral sensitization. A mixed Th1/Th2 response was observed with increases in interleukins 4, 5 and 10 and interferon γ as well as IgE, IgG1 and IgG2a. These responses were directed against Ara h 1 and Ara h 3 and, to a lesser extent, Ara h 2 and Ara h 6 [7]. In contrast to current thought that peanut allergy is primarily a Th2-driven process, the development of peanut sensitization seems to be more complex. The same group also examined the role of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), a negative regulator of T-cell activation, on peanut sensitization [8]. Naïve mice were orally exposed to peanut extract with or without cholera toxin in both the presence and absence of anti-CTLA-4 monoclonal antibody. Compared to mice treated with peanut extract and cholera toxin, those also given anti-CTLA-4 developed enhanced IgE responses to peanut extract and Ara h 1, 3 and 6, and increased mast cell degranulation following oral challenge. However, in the absence of cholera toxin, anti-CTLA-4 failed to elicit a similar allergen-specific response. The authors concluded

Curr Opin Allergy Clin Immunol 6:202–206. © 2006 Lippincott Williams & Wilkins.

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Current Opinion in Allergy and Clinical Immunology 2006, 6:202–206

Abbreviations

CPE	crude peanut extract
CpG	cytosine phosphate guanine
CTLA-4	cytotoxic T-lymphocyte-associated antigen 4
FAHF-1	Food Allergy Herbal Formula-1
HKLM	heat-killed <i>Listeria monocytogenes</i>

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1528-4050

that although CTLA-4 is not the critical factor in preventing sensitization, it does play a role in regulating the intensity of the sensitization response. In support of their previous work, it appears as though Th2 cytokine production alone is insufficient for the development of sensitization.

In addition to the progress made in the area of peanut sensitization, the same group of investigators examined the role of the food matrix on the immune response to peanut [9[•]]. This is important considering recent focus on the characterization and use of purified peanut allergens in immunotherapy, as will be discussed below. In one study, whole peanut extract injected into mouse popliteal lymph nodes induced a robust immune response as evidenced by increased cytokine production and increased antigen-presenting cells. In contrast, injection of purified peanut allergens failed to induce a similar response. The authors conclude that the food matrix influences the immune response and that purified peanut allergens possess little intrinsic stimulatory capacity.

Characterization of peanut allergens

To understand the immune response to peanut allergens and to develop potential therapies it is important to fully characterize specific peanut allergens. Although multiple peanut allergens have been identified (Ara h 1-8), recent focus has been on the three major allergens Ara h 1, Ara h 2 and Ara h 3, which have been characterized in most detail [10–12]. Ara h 1 and Ara h 2 appear to be more allergenic and are recognized by serum-specific IgE from approximately 70–90% of sensitized individuals [13]. Approximately 45% of peanut-allergic individuals have Ara h 3-specific IgE, and therefore it has been considered to play a lesser allergenic role [14]. However, in a recent study examining the major allergens in 16 Italian peanut-allergic children, 95% had IgE to Ara h 3, and in five of these individuals Ara h 3 was the only significant allergen detected [15]. Although it is difficult to draw such conclusions from a small sample size in one geographic area, Ara h 3 may be more allergenic than previously thought.

Although much is known about which allergens are most commonly recognized by IgE of peanut-allergic individuals, it is unclear how or if an individual's IgE-binding pattern relates to clinical severity. A recent study compared the IgE-binding patterns of 40 peanut-allergic individuals to symptom scores following challenge [16[•]]. Although there was no association between one particular peanut protein and challenge score, individuals who bound a higher number of peanut proteins had more severe clinical manifestations. This study also showed a positive association between protein bands corresponding to subunits of Ara h 3/4 and challenge score, further challenging the lesser role of Ara h 3.

A similar relationship between IgE-binding patterns and clinical severity was also demonstrated by Shreffler *et al.* [17[•]] in a study using sera from peanut-allergic subjects and microarray immunoassay to map IgE-binding epitopes of Ara h 1-3. Patients with a history of more severe reactions recognized a greater number of epitopes despite having similar serum-specific IgE to recombinant peanut allergens. These findings must be considered when developing immunotherapy using a single purified allergen and in selecting proteins for removal in an attempt to engineer a hypoallergenic peanut.

Therapeutic modalities for peanut allergy

As the immunologic response to peanut and the characteristics of peanut allergens have been further elucidated, opportunities for therapeutic intervention have become apparent. These therapeutic options are important because traditional immunotherapy using peanut extract, although effective, is associated with an unacceptable rate of systemic reactions [18]. In one study of rush immunotherapy using aqueous peanut extract, only three out of 12 patients tolerated the full maintenance dose [19]. The remainder of subjects required dose reductions and had partial or complete loss of protection as demonstrated by food challenge and skin-prick testing. Therefore, various other treatments are being explored. These include (1) traditional Chinese medicine, (2) immunotherapy using allergen peptides, mutated proteins and homologous allergens, (3) adjuvants for modified immunotherapy including immunostimulatory DNA sequences and heat-killed *Listeria monocytogenes* (HKLM), (4) plasmid DNA immunization and (5) anti-IgE therapy.

Traditional Chinese medicine

The Chinese herbal formula, Food Allergy Herbal Formula-1 (FAHF-1), contains a mixture of 11 herbs which are thought to contain anti-allergic properties. This herbal preparation has been used in Asia for many years and is now being explored in the USA for use in asthma and food allergy. After peanut-sensitized mice were treated with a 7 week regimen of FAHF-1, they developed no symptoms following peanut challenge and had significantly reduced histamine release and mast cell degranulation [20]. The treated mice demonstrated decreased peanut-specific IgE formation and decreased synthesis of interleukins 4, 5 and 13. A subsequent study used a simplified formula which contained only nine of the original 11 herbs. This formula, FAHF-2, was also effective in preventing peanut anaphylaxis in peanut-allergic mice [21[•]]. In both studies there were no observed toxicities, even at very high doses. It is currently unknown whether this formula could be simplified further or if it is as safe and efficacious in humans.

Modified immunotherapy

Because traditional immunotherapy using peanut extract is associated with an unacceptable rate of systemic reactions, modified forms of immunotherapy are being considered. One modified form, peptide immunotherapy, is based on the idea that allergen peptide fragments lack the ability to crosslink IgE on mast cells and basophils but will still interact with T-cells since T-cell epitopes are maintained. This was first explored in cat-allergic mice using peptides derived from Fel d 1 [22]. Subsequent small clinical trials in cat-allergic subjects showed potential of this method in the treatment of cat allergy [23,24].

To develop a T-cell-targeted peptide vaccine for peanut allergy it is important to characterize the T-cell epitopes of the major peanut allergens. Glaspole *et al.* [25] recently accomplished this by identifying two highly immunogenic T-cell epitopes of Ara h 2. The two regions, Ara h 2 (19–47) and Ara h 2 (73–119), induced significant T-cell proliferation and a Th2 cytokine profile in T-cell lines from peanut-allergic subjects. Furthermore, one study examined the effect of pepsin digestion of peanut extracts using sera from peanut-allergic subjects [26]. Multiple IgE-reactive proteins in CPE were eliminated after digestion with pepsin while T-cell reactivity was maintained. The clinical applicability of this concept in peanut-allergic patients remains to be determined.

A second form of modified immunotherapy utilizes peanut allergens that have been altered to decrease IgE binding, otherwise known as mutated protein immunotherapy. For example, single-amino-acid substitutions in the major IgE-binding epitopes of Ara h 1, 2 and 3 lead to a decrease or loss of IgE binding [10,11,27]. As a result, higher doses of allergens could be given more safely, making these modified recombinant allergens good candidates for use in peanut immunotherapy. Furthermore, T-cell epitopes are maintained, as is the ability to stimulate peanut-specific T-cells [28,29]. In addition to showing that modified Ara h 2 binds less IgE and still interacts with T cells, a recent study also looked at the ability of this modified allergen to release mediators from a rat basophil leukemia cell line [30]. This cell line was passively sensitized with IgE from peanut-allergic subjects. Compared with wild-type Ara h 2, these cells released significantly less β -hexosaminidase after exposure to modified Ara h 2. In light of these findings, the use of modified recombinant allergens in immunotherapy seems promising. However, as previously mentioned, the effect of the food matrix on the allergenicity of these modified peanut allergens must be considered. Furthermore, the T-cell signaling responses to these modified allergens needs to be explored and may uncover opportunities for modulation of the Th2 response to a Th1 response.

The possibility exists that the peanut-allergic response could also be modulated using homologous proteins. This concept has been used in birch-allergic individuals who have cross-reactive apple allergy, and it seems to be effective [31]. Soybean seed storage proteins share homology with peanut allergens and are thus candidates for immunotherapy in peanut-allergic individuals. One study desensitized peanut-allergic mice with CPE, soybean extract or placebo over a 4 week period [32^{*}]. The mice were subsequently challenged with CPE. Compared to sham-treated mice, there was a significant reduction in post-challenge symptom scores of both the peanut- and soy-treated mice. Furthermore, mice treated with soy had significantly lower peanut-specific IgG1 (a Th2-associated cytokine) compared to the peanut group. Both groups had higher levels of interferon γ and tumor necrosis factor α compared to placebo. While the use of homologous protein immunotherapy for peanut allergy seems promising, further studies need to explore its effects on peanut-allergic patients who have preexisting tolerance to soy.

Adjuvants for modified peanut immunotherapy

Certain DNA sequences have been shown to have immunostimulatory effects and to induce a Th1 response, especially those containing unmethylated cytosine phosphate guanine (CpG) motifs [33]. For this reason, CpG oligodeoxynucleotides could be used as potential adjuvants in immunotherapy. This was explored in a study using hen's egg lysozyme coadministered with CpG oligodeoxynucleotides to Th1- and Th2-biased mice [34]. In contrast to the Th2 cytokine pattern observed in response to hen's egg lysozyme alone, coadministration of CpG oligodeoxynucleotides resulted in a Th1-dominated cytokine pattern. Another study observed the effects of CpG oligodeoxynucleotides on peripheral blood mononuclear cells from allergic and nonatopic individuals [35]. CpG motifs induced interferon γ production by natural killer cells in both groups, and this was shown to be dependent on interleukin-12 production by dendritic cells. Furthermore, atopic individuals demonstrated increases in polyclonal and allergen-specific IgG and IgM and suppression of total IgE.

HKLM has been shown to promote a Th1 response as well [36]. Li *et al.* [37] showed that coadministration of HKLM with modified recombinant Ara h 1, 2 and 3 to peanut-sensitized mice led to decreased post-challenge symptom scores and reduced plasma histamine and peanut-specific IgE levels [37]. Furthermore, splenocytes from these mice produced less interleukin-5 and interleukin-13 and more interferon γ compared with sham-treated mice and mice treated only with modified peanut allergens.

A recent study used a dog model to examine the effects of peanut immunotherapy with HKLM on peanut-induced

anaphylaxis [38^{*}]. The dogs received one subcutaneous injection of HKLM mixed with peanut extract. Following vaccination, the oral dose of peanut required to elicit symptoms during challenge significantly increased in all dogs. There were significant reductions in skin test end-point titrations for over 10 weeks following treatment and reduced levels of Ara h 1-specific IgE. Thus, in both rodents and large mammals, HKLM appears to be a useful Th1-promoting adjuvant. Certainly, using these adjuvants in combination with modified recombinant allergen immunotherapy may be an option if, as suggested by recent work, the removal of the food matrix significantly decreases the immune-stimulating capacity of purified peanut allergens.

Plasmid DNA immunization

Immunization with plasmid DNA encoding a specific antigen results in uptake by antigen-presenting cells and synthesis of the encoded antigen. In animal models this has been shown to promote a Th1 response and therefore might be useful in modified immunotherapy. Antibody production after plasmid DNA immunization consists of predominantly IgG2a (a Th1-associated cytokine) whereas protein immunization without plasmid DNA induces IgG1 and IgE (Th2 cytokines) [39]. In one study, rats receiving plasmid DNA encoding the house dust mite allergen, Der p 5, had decreased airway hyperresponsiveness to allergen challenge and decreased histamine release into bronchoalveolar fluid [40]. Similar findings have been reported using plasmid DNA encoding ovalbumin in mice [41]. Hengge *et al.* [42] showed that plasmid DNA is taken up and genes are expressed in human skin grafts on immunocompromised mice. Thus plasmid DNA immunization may represent a potential form of immunotherapy for peanut allergy, although direct studies examining this are still lacking.

Anti-IgE therapy

TNX-901 is a humanized monoclonal antibody against the Fc portion of IgE. Thus it prevents binding of IgE to FcεR1 on mast cells and basophils, and downregulates FcεR1 expression on these cells. Leung *et al.* [43] examined 84 peanut-allergic individuals in a randomized, double-blind trial of patients receiving TNX-901 or placebo. Patients treated with the highest dose of TNX-901 (450 mg) had a significant increase in their oral food challenge threshold after four doses. The threshold increased from approximately half a peanut to nine peanuts following treatment. Some subjects on the active therapy had no change from the baseline in the their threshold to cause a positive reaction to peanuts. Although this treatment would not eliminate peanut sensitivity, it would be protective in cases of accidental ingestion. However, treatment with TNX-901 would have to be continued indefinitely to maintain protection,

and may be more useful in conjunction with other therapeutic modalities.

Conclusion

Peanut allergy is among the most serious, life-threatening food sensitivities and is complicated by its usual persistence and high risk of accidental exposures. For this reason, much effort has been put forth to further elucidate the immunologic response to peanut and in the characterization of peanut allergens. This knowledge has given rise to many possibilities in the treatment of peanut allergy, and an alternative, safer form of immunotherapy for peanut allergy will likely utilize a combination of these. Further studies are needed to explore the safety and effectiveness of these treatments in humans.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 245).

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