



Doctor, can I desensitize my food-allergic child using directly the allergenic molecules?

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Purpose of review

Food allergy has risen in prevalence worldwide and is one of the main causes of anaphylaxis, especially in children. The only possible therapeutic approach is specific immunotherapy. This review describes the recent approaches using allergenic molecules for specific immunotherapy.

Recent findings

Hypoallergenic tropomyosins from *Metapenaeus ensis* have been cloned and constructed by direct mutagenesis or epitope deletion and have been successfully used in shrimp-sensitized mice. A modified carp parvalbumin is being used in phase I/IIa clinical trials in patients with fish allergy. Natural lipid transfer protein (Pru p 3) and profilin (Pho d 2) extracts have been used for the treatment of patients with plant food allergy. Treatments of egg-sensitized mice with glycosylated-ovalbumin and ovomucoid peptides led to a clinical and immunological improvement. A preventive treatment with synthetic β -lactoglobulin peptides was effective in reducing skin symptoms in mice sensitized to milk whey proteins but no dominant epitopes were found in α -lactalbumin. Finally peanut desensitization has been attempted using three modified recombinant peanuts but the protocol was interrupted and limited to a phase 1 study because of side-effects.

Summary

Many molecules, including allergenic peptides or modified proteins are under consideration but clinical trials in food-allergic study participants are necessary to confirm tolerability and efficacy.

Keywords

allergenic molecules, desensitization, food allergy, specific immunotherapy

INTRODUCTION

Food allergy is defined as an adverse, reproducible immune-mediate response to a given food. The term allergy encompasses both IgE-mediated and non-IgE-mediated reactions.

Every food can be responsible for an allergic reaction but the great majority of allergic reactions are provoked by peanut, tree nuts, seafood, cow milk, egg, wheat, soy, fruits/vegetables (often because of cross-reactivity with pollens), and seeds. According to various studies, food allergy affects about 5% of adults and 8% of children in western countries and its prevalence has increased in the last few decades. Cow milk, egg, peanut, tree nuts, soybean, wheat, and seafood are the main responsible of food allergy in children. In adults, peanut, tree nuts, seafood, and fruit/vegetables are the most important food allergens [1,2].

The rate of IgE-mediated allergy resolution varies according mainly to the food and the patient's age (milk and egg allergy have a higher resolution rate than peanut within the first 5 years of

life) [3]. Current management of food allergy is based on food avoidance and recognition and treatment of allergic reactions but food avoidance is not always possible because of the presence of hidden allergens and may affect patients' quality of life and/or their nutritional status. For these reasons since the 1980s researchers have paid attention to oral food-specific immunotherapy (SIT) to treat food-allergic patients [4]. The exact mechanisms of food immunotherapy are still not known but immunologic changes include decreased reactivity of mast cells and basophils, increased food-specific IgG4 and increased regulatory T cells (Tregs). Tregs suppress

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Curr Opin Allergy Clin Immunol 2016, 16:000–000

DOI:10.1097/ACI.0000000000000264

KEY POINTS

- SIT is the only causative active treatment for food allergy.
- Single allergenic molecules have been identified for most foods.
- Tolerogenic peptides and modified allergenic molecules have been produced to reduce side-effects of food-SIT.
- These molecules showed clinical efficacy and low side-effects in many mouse models of food allergy.
- Clinical trials in human study participants are now necessary before clinical use (a subcutaneous SIT with parvalbumin is undergoing a phase I/IIa clinical trial).

Th2-immune response via secretion of IL-10 and/or TGF β . The development of Tregs is responsible for permanent tolerance (or long-term desensitization) [5[¶]].

The great majority of published studies deal with food oral immunotherapy (OIT) with whole foods but recently some authors have paid attention also to sublingual immunotherapy (SLIT) and epicutaneous immunotherapy. Subcutaneous immunotherapy (SCIT) has been attempted in the past but has been abandoned because of side-effects [6–12].

The purpose of this review is to provide an update on the possibility to use recombinant or native major allergenic molecules to treat food allergic patients.

SEAFOOD ALLERGY

Shellfish allergy has a worldwide prevalence of about 0.6%, is the second commonest food sensitivity in the United States and is highly prevalent in Asian countries [13,14]. Anaphylaxis is frequently reported. The muscle protein tropomyosin is the major shrimp allergen in both *Penaeus* and *Metapenaeus* spp. It is a 34–38 kDa which is involved in contractile activities of muscle cells. Because of its homology sequence among crustaceans and mollusks, tropomyosin is considered to be the major cross-reactive shellfish panallergen. Way *et al.* cloned and expressed tropomyosin from *Metapenaeus ensis* (Met e 1) which showed clinical reactivity from sera of shrimp allergic patients. They firstly tried to identify the major IgE-binding epitopes and then to reduce the allergenicity of the molecule by introducing point mutations within the IgE-binding epitopes or deleting these epitopes. Sera from 12 patients with shrimp allergy had a high IgE-binding activity against five peptides. Two

mutant tropomyosins were then constructed by direct mutagenesis (MEM49) or epitope deletion (MED171). Both tropomyosins showed decreased IgE-reactivity *in vivo* when using sera from shrimp allergic patients and Met e 1-sensitized mice. As MEM49 and MEM171 CD4⁺ T cell epitopes were not modified, hypoallergenic tropomyosins maintained the capability to induce an IgG response. Hence these molecules may be used with fewer side-effects when compared with the native proteins [15[¶]].

The same authors also tried to identify T-cell epitopes on Met e 1 tropomyosin which differs from Pen a 1 by one amino acid position. Eighteen peptides spanning the full length of Met e 1 were synthesized and six were identified as major epitopes by spleen cell stimulation of sensitized mice. Sensitized mice treated with a mixture of the six identified T-cell epitopes showed a reduction in the allergic response, an increase of serum-specific IgG_{2a}, the induction of Tregs and a switch from a Th2 to a Th1 response. The authors concluded peptide immunotherapy may be applied in humans for the treatment of shrimp allergy. Both studies provide evidence of the possibility of different therapeutic approaches but studies in human participants are mandatory to confirm these results [16^{¶¶}].

Fish allergy has an estimated prevalence of 0.1% in Europe when assessed by food challenges and may present with severe symptoms, including anaphylaxis [17].

Parvalbumins are the major fish allergens. They are calcium-binding muscle proteins of 10–13 kDa which are resistant to heat, denaturation, and proteolysis. Gad c 1 from codfish was the first parvalbumin identified and characterized. Carp parvalbumin, Cyp c 1, has high cross-reactivity with other fish parvalbumins and a recombinant version with reduced IgE-binding activity by modifying the two active Ca²⁺-binding sites has been produced. The Food Allergy-Specific Immunotherapy project chose this modified protein to develop an alum-adsorbed parvalbumin vaccine for SCIT of fish allergy [18,19]. Natural parvalbumin, nCyp c 1, was obtained by carp muscle whereas a recombinant wild-type parvalbumin rCyp c 1 and the mutant parvalbumin mCyp c 1 were obtained as previously described. mCyp c 1 showed a secondary structure very similar to the wild-type allergen and a lesser ImmunoCAP inhibition capacity when compared with both nCyp c 1 and rCyp c 1. Also the basophil activation test showed that mCyp c 1 had a reduction of allergen activity. rCyp c 1 and mCyp c 1 were both able to induce an IgG response in mice and proliferation of peripheral blood mononuclear cells isolated from sera of fish-allergic patients.

Toxicity studies performed in mice and rabbits of alum-adsorbed mCyp c 1 did not show any clinical sign of toxicity at doses 10 000 and 300 times higher than the doses that will be used for the first clinical trial in human study participants [20[¶]].

These preclinical studies provide evidence that hypoallergenic molecules have lesser IgE-binding activity resulting in reduced side-effects but maintain the capability to interact with the immune system.

A phase I/IIa clinical trial (NCT02017626) involving 16 fish-allergic study participants using mCyp c 1 was conducted in Denmark from September 2013 to July 2014, but researchers encountered some difficulties in enrolling sufficient study participants. Preliminary data showed low side-effects and a good immunological response and so an extension of the project has been obtained (data have not been published yet) [19].

PLANT FOOD ALLERGY

Plant food allergy is very common in older children and adults, tree nuts and fruits being among the most important food allergens [1]. Lipid transfer proteins (LTPs) are small highly conserved molecules which are responsible for *Rosaceae* food allergy in the Mediterranean area. They are plant panallergens resistant to proteolysis, to pH changes and to heat and for these reasons may cause symptoms ranging from the involvement of the oropharyngeal tract to anaphylaxis. Particularly LTP sensitization is frequently involved in food-dependent anaphylaxis. Symptoms may be provoked by the concomitant administration of NSAIDs or alcohol ingestion.

Studies [21,22] in Spain and Italy demonstrated peach as the primary sensitizer in the Mediterranean basin. For these reasons some Spanish authors attempted sublingual desensitization in peach allergic patients using a peach extract obtained from fresh peelings and quantified in micrograms of Pru p 3, the LTP of peach. These authors showed that a 6-month period of SLIT with a peach extract led to an immunological and clinical improvement, as confirmed by a decrease in skin prick test, an increase in specific IgE and IgG4 to Pru p 3 and a higher tolerance of peach assessed by double-blind placebo-controlled food challenge. No significant differences were observed in the placebo group. No serious adverse events were observed in the active group. Reactions consisted mainly in oral local reactions which receded with oral antihistamines [23].

After these pivotal attempts of LTP immunotherapy, the Food Allergy-Specific Immunotherapy

project aimed to develop and produce, according to good manufacturing practice, a modified LTP to be used for SLIT. Five candidate molecules were tested but two failed on hypoallergenicity, two on immunogenicity and one on solubility. The loss of allergenicity led to reduced immunogenicity and stability. This was probably because of the disruption of the tertiary structure of the molecule. Also Fra a 3, the strawberry LTP, 80% homologous to Pru p 3, which was thought to be a natural hypoallergen, was abandoned because of the lack of immunogenicity in animal models [18,19]. So LTP standardized extracts are not available and are not under evaluation for SCIT.

Profilins are highly conserved proteins which are frequently involved in cross-reactivity between vegetable food and pollens. Profilins are denatured by both heat and pepsin digestion and for these reasons they provoke symptoms that are usually limited to the proximal gastrointestinal tract (the so-called oral allergy syndrome). However, some authors found profilins may cause also systemic symptoms in sensitized patients exposed to high amounts of pollens during the pollen season [24]. Moreover, Asero *et al.* [25] recently found 23% (296 out of 1276) pediatric patients are sensitized to profilin and 33% of them reported typical oral allergy syndrome symptoms after plant-derived foods.

For these reasons some researchers attempted a SLIT using a palm profilin extract (containing 50 µg/ml of Pho d 2) in seven patients with oral allergy syndrome after ingestion of vegetable foods such as tomato, peach, eggplant, zucchini, watermelon, melon, and so on. At the end of the treatment all patients showed a better tolerance of foods as demonstrated by double-blind placebo-controlled food challenges and reduction of Pru p 4-specific IgE. This pilot study [26[¶]] gives evidence SLIT for profiling desensitization may be effective and with no side-effects, but larger studies are necessary to determine optimal duration of treatment and dosage regimen.

EGG ALLERGY

Egg allergy is the second most frequent food allergy in children. About 50% of affected children outgrow egg allergy at the age of 72 months [27]. The egg white contains more allergenic proteins than the yolk. Ovomucoid (OVM) (Gal d 1), ovalbumin (OVA) (Gal d 2), ovotransferrin/conalbumin (Gal d 3), and lysozyme (Gal d 4) are the major egg allergens. α-livetin (Gal d 5) is the major allergen of yolk. OVM is stable to heat and peptic digestion and for these reasons is the most allergenic egg protein. Oral-specific desensitization has been used to treat egg-

allergic patients using natural raw or cooked whole egg, dehydrated whole egg or egg white, liquid pasteurized whole egg or egg white, and lyophilized whole egg with different rates of success ranging from 48 to 100% of treated patients [28].

Oral immunotherapy using the dominant T-cell epitope of egg OVM showed to be effective in reducing clinical symptoms in orally sensitized mice to OVM. The authors isolated and used the single peptide 151–171 of OVM and a multiple peptide (three repeated single peptide linked by alanine residues). Specific OVM-IgE showed a significant decrease in both groups treated with the single or the multiple peptides. A significant increase of CD4⁺ FOXP3⁺ and CD4⁺ CD25⁺ cells was also detected [29].

Another approach is based on the conjugation of proteins with reducing sugars via the Maillard reaction to reduce allergenicity of molecules. A group of 60 Balb/c mice was sensitized with 1 mg of OVA adjuvanated with 10 µg of cholera toxin. Mice were then treated for 4 weeks with various glycosylated OVA forms (OVA-glucose, OVA-mannose, OVA-glucomannan, OVA-galactomannan, and OVA + a mixture of hydrolyzed glucomannan). Mice treated with OVA-mannose and OVA-glucomannan showed an improvement of clinical signs, a reduction of specific IgE and an increase of Treg cells [30²²]. The same authors confirmed mannosylated egg white proteins are able to attenuate the immune response in sensitized mice whereas mannosylated peanut and whey proteins did not show any immunological or clinical effect [31].

The above approach may lead to an effective treatment of egg allergy, especially in those patients who are sensitized to ovomucoid and are at higher risk of reaction also after consumption of cooked egg.

MILK ALLERGY

Milk allergy is the most frequent food allergy in children with an estimated prevalence of 1.6–3% in the first year of life and of less than 1% in children aged 6 years or more. Caseins and the whey proteins, α-lactalbumin (α-LA) and β-lactoglobulin (β-LG), are the main allergens of cow milk. About 60–75% of children affected by cow milk allergy develop tolerance within the fifth year of life [32]. Children who do not outgrow their allergy and are at risk of systemic reactions may benefit from SIT. Until now oral SIT has been attempted in several clinical trials [33].

T-cell epitopes have been identified for both α-LA and β-LG. A preventive treatment with synthetic β-LG peptides was effective in reducing skin symptoms in mice sensitized to whey proteins. The effect of treatment seems to be mediated by dendritic cells and Tregs. The authors also found that a diet rich in short-

chain galacto and long-chain fructo-oligosaccharides and pectin-derived acidic oligosaccharides enhances the efficacy of the treatment and it is probably because of an influence of the intestinal microbiota [34]. The same authors tried to identify T-cell epitopes in bovine α-LA. Nineteen synthetic peptides were investigated but no dominant epitopes were found in α-LA. The authors conclude that no peptides of α-LA can be used for immunotherapy [35²¹].

Several proteins are involved in the pathogenesis of cow milk allergy; therefore the possibility of using a single allergenic molecule seems to be still very far from clinical practice.

NUT ALLERGY

Peanut allergy is now the most common cause of fatal food-allergic anaphylaxis [36]. Ara h 1, Ara h 2, and Ara h 3 are the three major peanut allergens and most people are sensitized to at least one of them. Almost 90% of peanut-allergic patients are sensitized to Ara h 2 in North America. Ara h 2 and its homologue Ara h 6 are more able to induce basophil degranulation than Ara h 1 and Ara h 6. In contrast to milk and egg allergy, children suffering from peanut allergy outgrow their allergy in no more of 20% of cases. For this reason a therapeutic approach such as SIT is advisable [37].

The first clinical trials regarding subcutaneous immunotherapy with a peanut extract were soon abandoned because of side-effects [38]. The researchers paid attention to both SLIT and OIT, with good results and minor incidence of side-effects [39,40].

Pascal *et al.* identified four dominant regions in Ara h 2 that were able to induce T cell proliferation *in vitro*. The authors conclude these peptides may be used for SIT with a significantly lower risk of systemic reactions when compared with whole peanut extracts [41].

Some authors attempted peanut desensitization using three modified recombinant peanut proteins (Ara h 1, Ara h 2, and Ara h 3) encapsulated in the bodies of heat and phenol killed *E. coli*. The treatment was well tolerated by the control group but five out of 10 allergic patients experienced side-effects (including anaphylaxis in two cases). For this reason the protocol was interrupted and limited to a phase 1 study [42].

Plundrich *et al.* firstly demonstrated that polyphenol-fortified peanut matrices are able to reduce the allergenicity of peanut proteins as shown by a basophil degranulation assay and a reduction of IgE-binding of peanut proteins. Then they found peanut protein-polyphenol complexes were less resistant to pepsin digestion. Ara h 1, Ara h 2, Ara h 3, and Ara h 6 digestion occurred more rapidly in protein-

cranberry and protein-green tea polyphenol complexes than in protein-cinnamon polyphenol complex or in uncomplexed peanut flour. These studies provide evidence polyphenol-peanut protein complexes are less allergenic than native molecules and pepsin digestion produce less reactive peptides which maintain antigenicity. Thus this strategy may be useful to reduce side-effects during OIT with native allergens [43,44].

Pepsinized cashew proteins showed to maintain their antigenicity when compared with native proteins in a mouse model of cashew nut allergy. The authors treated cashew-sensitized mice with a native cashew protein extract or with a pepsin-digested cashew protein extract. Both extracts were able to induce an increase of specific IgG₁ and IgG_{2a} and a reduction of Th2 cytokines such as IL-13 and IL-5 but mice treated with the pepsin-digested extract showed reduced allergic reactions [45].

CONCLUSION

The deeper knowledge of food allergens and the need for well tolerated treatment are leading to the development of new approaches to food SIT. The great majority of the studies are focusing on allergenicity reduction modifying the native allergens. However, the loss of allergenicity has also led to a loss of antigenicity vanishing the efforts of scientists. Moreover, no large phase II/III trials have been conducted in human study participants. For these reasons we are still far away from the routine use of the allergenic molecules for food immunotherapy. SIT using whole foods or native proteins, with all its limitations regarding possible side-effects, duration regimens, and optimal doses, is actually the only active therapeutic option for food allergy.

Acknowledgements

None.

Financial support and sponsorship

Dr Alessandro Buonomo did not receive any funding for this work.

Conflicts of interest

There are no conflicts of interest.

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