

Engineering, Characterization and in vitro Efficacy of the Major Peanut Allergens for Use in Immunotherapy

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Key Words

Hypoallergenic · Allergens · Immunotherapy · Peanut allergy

Abstract

Background: Numerous strategies have been proposed for the treatment of peanut allergies, but despite the steady advancement in our understanding of atopic immune responses and the increasing number of deaths each year from peanut anaphylaxis, there is still no safe, effective, specific therapy for the peanut-sensitive individual. Immunotherapy would be safer and more effective if the allergens could be altered to reduce their ability to initiate an allergic reaction without altering their ability to desensitize the allergic patient. **Methods:** The cDNA clones for three major peanut allergens, Ara h 1, Ara h 2, and Ara h 3, have been cloned and characterized. The IgE-binding epitopes of each of these allergens have been determined and amino acids critical to each epitope identified. Site-directed mutagenesis of the allergen cDNA clones, followed by recombinant production of the modified allergen, provided the reagents necessary to test our hypothesis that hypoallergenic proteins are ef-

fective immunotherapeutic reagents for treating peanut-sensitive patients. Modified peanut allergens were subjected to immunoblot analysis using peanut-positive patient sera IgE, T cell proliferation assays, and tested in a murine model of peanut anaphylaxis. **Results:** In general, the modified allergens were poor competitors for binding of peanut-specific IgE when compared to their wild-type counterpart. The modified allergens demonstrated a greatly reduced IgE-binding capacity when individual patient serum IgE was compared to the binding capacity of the wild-type allergens. In addition, while there was considerable variability between patients, the modified allergens retained the ability to stimulate T cell proliferation. **Conclusions:** These modified allergen genes and proteins should provide a safe immunotherapeutic agent for the treatment of peanut allergy.

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Introduction

In North America and Europe a large portion of the population is affected by allergic reactions to common environmental proteins, making atopic disorders among

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the most prevalent diseases in the industrialized nations [1]. In the most severe cases, exposure to an allergen in sensitive individuals can produce a life-threatening anaphylactic response. For example, allergy to peanuts is the most common single cause of anaphylaxis outside of hospitals in the United States [2, 3].

Documented food hypersensitivity reactions affect approximately 6–8% of children and 1–2% of adults [4, 5]. Peanuts, milk and eggs account for the majority of reactions in children, while peanuts, tree nuts and shellfish are responsible for the majority of reactions in adults [6]. Most children will outgrow allergies to foods such as milk, eggs, wheat and soybean. Unfortunately, allergy to peanuts will typically persist into adulthood, lasting the entire lifetime of the individual. It is estimated that about 0.6% of the United States population is allergic to peanuts [7].

Despite the prevalence of peanut hypersensitivity in the population, and an increasing number of deaths each year from peanut-induced anaphylaxis, there are no therapeutic options available to prevent life-threatening allergic reactions to peanut. The only treatment available for peanut-hypersensitive patients is strict avoidance of peanut allergens [2]. Recently, the food industry has started using peanuts as a supplemental protein source in a wide variety of processed foods [8]. This inclusion of potential hidden peanut allergens makes accidental consumption almost unavoidable for peanut-sensitive individuals. In fact, in a retrospective study of peanut-induced clinical symptoms, 50% of allergic individuals suffered a peanut-induced hypersensitivity reaction in a 2-year period [9].

In this communication, we describe the characteristics of hypoallergenic variants of the major peanut allergens Ara h 1, Ara h 2 and Ara h 3 that exhibit properties favorable for allergen immunotherapy. By modifying the allergen cDNAs to express proteins with reduced IgE-binding capacity, it was possible to produce hypoallergenic variants. These variants were shown to be poor competitors for serum IgE from a pool of peanut-hypersensitive patients; however, they retained the ability to stimulate T cell proliferation. In addition, desensitization of a murine model of peanut allergy with the modified Ara h 2 protein resulted in reduced amounts of Ara h 2-specific IgE and significantly lowered anaphylaxis scores when compared to controls. The modified peanut allergen genes and proteins could provide a safe immunotherapeutic agent for the treatment of peanut allergy.

Results

The ability of the modified peanut allergens to bind IgE from individual patients was determined by immunoblot analysis. Serum IgE from peanut-sensitive patients was incubated with immunoblot strips containing equal amounts (2 µg) of the wild-type and modified proteins and the amount of IgE bound to each as determined as described above. Individual patient serum IgE binding to the modified protein was expressed as a percentage of IgE binding to the wild-type allergen. There were 57 patients tested for IgE binding to Ara h 1, 52 patients for Ara h 2, and 52 patients for Ara h 3. The range of IgE binding to modified allergens was from 5.4–125% of wild-type Ara h 1 allergen, 0–99.8% of wild-type Ara h 2 allergen and 19.3–141% of wild-type Ara h 3 allergen. The average decrease in IgE binding to the modified allergen in the current population of peanut-sensitive patients was 35% for Ara h 1, 71% for Ara h 2 and 41% for Ara h 3.

To examine the ability of the modified allergens to continue to interact with T cells from peanut-sensitive individuals, peripheral blood mononuclear cells (PBMC) were incubated in the presence of these proteins and proliferation was assessed by the incorporation of radioactive thymidine into the DNA of dividing cells. PBMCs from 12 peanut-sensitive individuals were tested for each wild-type and modified allergen and the stimulation index for the modified allergen was expressed as a percentage of that obtained for the wild-type allergen. The range of stimulation indexes for the recombinant wild-type allergens was 2–28.7 for Ara h 1, 2–12.7 for Ara h 2 and 2–26.3 for Ara h 3. In comparison, the average stimulation index produced by the modified allergens in relation to its wild-type counterpart was 72% for Ara h 1, 104.3% for Ara h 2 and 72.2% for Ara h 3. These results indicate that the modified allergens retained the ability to interact with T cells.

Utilizing a murine model of peanut anaphylaxis [10], wild-type Ara h 2 was used to first sensitize mice, and then desensitize the mice with either wild-type Ara h 2 protein or the modified Ara h 2 protein and monitor histamine release and clinical symptoms. As expected, PBS-sham-treated mice exhibited significantly higher clinical symptom scores when compared to either of the experimental groups. While the mice treated with the wild-type Ara h 2 protein appeared to experience reduced hypersensitivity symptoms compared to controls following challenge, the mice desensitized with modified Ara h 2 had significantly lower clinical symptom scores than either of these groups. Furthermore, to determine whether the

reduced clinical symptom scores in the modified Ara h 2 protein-treated mice reflected a reduced histamine release, we measured plasma histamine levels following challenge. Histamine levels were markedly increased in sham-treated mice compared to naïve mice and in the modified Ara h 2-treated groups.

Discussion

Our results suggest that it is possible to engineer a hypoallergenic protein that fulfills important criteria for use as an immunotherapeutic agent for allergic disease. A lowered capacity to bind IgE allows higher doses of allergen to be used, a criterion for successful immunotherapy [11], with lowered probability of life-threatening side effects. Retention of the allergen's ability to interact with T cells allows for the shift from an allergen-specific Th2 response to an allergen-specific Th1-type response. By injecting larger quantities of modified peanut allergens, there is a better chance that these proteins would be pro-

cessed by nonprofessional APCs. If processed peptides are presented to circulating T cells by APCs without the proper costimulatory molecules, then T cells will become desensitized to that T cell epitope [12]. Desensitization of the T cell results in a decrease in IL-4 production, resulting in a decreased capacity to activate B cells to produce peanut allergen-specific IgE. Since the modified peanut proteins displayed a decreased capacity to bind serum IgE and retained the ability to stimulate T cell proliferation from PBMCs donated by peanut-allergic patients, the modified proteins have the potential to be useful in a clinical setting for allergen-specific immunotherapy aimed at treating peanut-hypersensitive patients.

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