

Modification of a Major Peanut Allergen Leads to Loss of IgE Binding

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Key Words

Peanut allergen · Hypoallergenic · IgE · Immunotherapy

Allergy to peanut is a significant IgE-mediated health problem because of the high prevalence, potential severity, and chronicity of the reaction. Currently the only treatment for peanut hypersensitivity is avoidance of the food which is inadequate as shown by the fact that 50% of peanut-sensitive individuals in a recent study [1] reported a significant accidental reaction within a 2-year period. Therefore, the goal of our laboratory is to develop specific, safe, and effective immunotherapy for patients suffering from peanut hypersensitivity. A potentially safe and efficacious immunotherapeutic strategy would be one that utilized allergenic proteins modified in such a way so they no longer bind IgE but still could bind T cells and either elicit a Th₁ type response or lead to tolerance. Ara h 2, an abundant peanut protein, is recognized by serum IgE from >90% of peanut-sensitive individuals [2]. It has been shown to belong to the conglutin family of seed storage proteins and contain 10-linear IgE-binding epitopes three of which were immunodominant [3]. Our previous work has shown that individual epitopes, when taken out of the context of the intact allergen, could be mutated to non-IgE-binding sites by changing a single amino acid to alanine [3]. We wanted to take this concept one step further and produce a gene that had the immunodominant epitopes changed to non-IgE-binding sites, produce this modified version of the allergen

and determine by immunoblot analysis the extent that the overall allergenicity of the protein had been reduced.

Amino acids important for IgE binding in epitopes 3, 4, 6, and 7 of the Ara h 2 gene were mutated to encode alanine by single-stranded mutagenesis or by PCR mutagenesis of the full length Ara h 2 cDNA clone. Mutations were confirmed by DNA sequence analysis of the modified Ara h 2 clone. The wild-type and modified Ara h 2 cDNA inserts were directionally cloned into the procaryotic expression vector pET24 plasmid. This vector encodes a T7-tag at the N-terminus and a 6X HIS tag at the C-terminus of expressed proteins. Exponentially growing bacterial cells carrying the wild-type or modified Ara h 2 clones were induced to express the recombinant proteins with 1 mM IPTG. Cells were pelleted and recombinant Ara h 2 proteins were purified via their HIS tag by affinity chromatography on a nickel resin column.

The recombinant wild-type and modified Ara h 2 proteins were assayed for their ability to bind IgE, bind IgG and to stimulate peanut-specific T cell lines to proliferate. Immunoblot analysis was performed using serum from peanut-sensitive individuals and wild-type and modified recombinant proteins to determine the effect that single amino acid substitutions in epitopes 3, 4, 6, and 7 had on IgE and IgG binding. In 12/16 peanut-sensitive patients tested there was a dramatic decrease in IgE binding when compared with binding to the wild-type control. Three of the patients showed similar amounts of IgE binding to both wild-type and modified allergen while 1 patient showed an increase in binding to the modified allergen. There was no discernable

difference in IgG binding to the modified allergen when compared to the wild-type protein. In addition, Ara h 2-specific T cell lines developed from peanut-sensitive patients were stimulated to proliferate to the same degree whether the modified or wild-type allergen were used. These results indicate that it is possible to develop a hypoallergenic protein that retains the ability to bind IgG and stimulate T cell proliferation, confirming the possibility that a safe efficacious immunotherapy may be developed for the treatment of peanut allergy.

Acknowledgements

This work was supported by grants from the National Institute of Health RO1-AI-133596 and the Clarissa Sosin Allergy Research Foundation.

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