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Clinical reactivity to raw peanut correlates with IgE binding to conformational epitopes of Ara h 1: a Case Report

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Most peanut allergic individuals have reactivity to the dominant allergens, Ara h 1, 2 or 3, and a limited number of that promote IgE of responses (1). While IgE recognition of aeroallergens is frequently against conformational epitopes, the responses to peanuts, and foods in general, is toward linear epitopes (2). Conformational epitopes to foods, mainly egg or milk, are actually associated with development of tolerance (3). Conversely, reactivity to linear epitopes is associated with food allergy persistence (4). Here, we report data on an unusual case of peanut allergy that supports IgE-mediated clinical reactivity to conformational epitopes in Ara h1.

We show specific IgE reactivity to Ara h1 in peanut that is because of conformational epitopes.

A 35-year-old male patient with a clinical history of chronic nasal congestion, intermittent postnasal drip, ocular pruritus increased in Spring and Fall or with cat exposure, and oral pruritus to several foods presented for allergic evaluation. The oral pruritus, similar in presentation to oral allergy syndrome (OAS), was often accompanied by tongue swelling but not with difficulty breathing and occurred with uncooked tree nuts and uncooked peanuts. He had similar symptoms in the past with banana. Surprisingly, he reported not having these symptoms when eating food products in which the peanuts had been cooked. He was found to be highly reactive upon skin testing to the following allergens: peanut, brazil nut, almond, hazelnut, pecan, pistachio, and pine nut, as well as trees, grasses, ragweed, mold, house dust mite and cat, but negative to cashew and walnut. He was found to have a peanut-specific IgE titer of 5.5 kU/L.

To test whether reactivity was being lost upon heating, whole peanut extract was heated to boiling and immunoblotted using the patient's serum. IgE reactivity was detected using HRP-conjugated anti-human IgE. Oral allergy syndrome has been reported to associate with reactivity toward the relatively unstable 16- to 17-kD-sized Ara h 8 protein (5). While we did observe a heat-affected protein at this size, as well as an additional band at approximately 20 kD, Fig. 1A demonstrates that the reactivity to another specific band at approximately 60 kDa was rapidly lost upon boiling, while most others were unaffected. To identify this protein, we performed two-dimensional protein analysis on raw, whole peanut extracts and resolved this into a number of potential protein spots (Fig. 1B). Six potential proteins were selected for mass spectrometry-based identification based on immunoblotting of the protein map using the patients serum and included one protein of a size that was unaffected by boiling (Fig. 1B). The protein identities showed that spots 2–6 all contained the major peanut allergen Ara h 1 while protein spot 1 was Arachin 6. We utilized a peptide

array-based immunoassay of sequential linear epitopes for Ara h1, 2 and 3, as we have previously described and shown to correlate with symptom severity (1) and found no IgE recognition of linear epitopes of Ara h1, 2 or 3.

Thermal processing has been the subject of a number of studies, in large part because of the dietary differences in peanut consumption between westernized and other societies. Some report reductions in the IgE-binding capacity of several peanut proteins, including Ara h 1 (6, 7). However, these studies show relatively small reductions and only after long periods of boiling (>20 min). This response is highly suggestive of a conformational epitope or reactivity toward a secondary modification, such as glycosylation or phosphorylation. However, two-dimensional resolution discriminates proteins with different modifications and because the patient's IgE showed reactivity toward six different modifications of Ara h 1, it is most likely that a conformational epitope is involved.

In conclusion, we report a patient with novel form of peanut allergy, wherein IgE reactivity is rapidly lost upon cooking and symptoms are dominated by oral pruritus. We demonstrate that this reactivity is to Ara h 1 but believe it is against a conformational epitope, which has not been reported before for peanut allergens.

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Figure 1.

(A) IgE reactivity to peanut proteins is rapidly lost upon boiling. Whole peanut protein was boiled for the indicated times and immunoblotted using the patient's sera. Anti-human IgE was used to detect IgE reactivity. (B) Two-dimensional analysis of peanut extract demonstrating multiple reactive protein spots. Whole peanut protein was separated by size and charge using two-dimensional electrophoresis, and the separated proteins determined by Coomassie blue-stained protein map and immunoreactivity by immunoblot using patient serum and anti-IgE. Circles represent the protein spots that were sequenced.