

Anaphylaxis to Peanut in a Patient Predominantly Sensitized to Ara h 6

A. Asarnoj^{a, b, d} S. Glaumann^{c, e} L. Elfström^f G. Lilja^{c, e} J. Lidholm^f
C. Nilsson^{c, e} M. Wickman^{a, c, d}

^aNational Institute of Environmental Medicine, Karolinska Institutet, ^bDepartment of Paediatrics, Astrid Lindgren's Children's Hospital, ^cDepartment of Paediatrics, Sachs' Children's Hospital, ^dCentre for Allergy Research, Karolinska Institutet, and ^eDepartment of Clinical Science and Education, Södersjukhuset, Karolinska Institutet, Stockholm, and ^fThermo Fisher Scientific (formerly Phadia AB), Uppsala, Sweden

Established Facts

- The peanut allergen component Ara h 2 is considered to indicate true peanut allergy. Another peanut 2S albumin, designated Ara h 6, cross-reacts extensively with Ara h 2.

Novel Insights

- In rare cases, Ara h 6 sensitization may occur in the absence of Ara h 2 sensitization and cause a severe reaction to peanut on exposure.

Key Words

Anaphylaxis • Food allergy • Peanut • Ara h 2 • Ara h 6

Abstract

Diagnosis of peanut allergy has improved thanks to component-resolved diagnostics. Peanut allergen component Ara h 2 is considered to indicate true peanut allergy. The component Ara h 6 is structurally similar to Ara h 2, but the diagnostic value of analyzing IgE antibodies to Ara h 6 is unclear. A boy sensitized (≥ 0.35 kU_A/l) to Ara h 8 but not to Ara h 1, Ara h 2 and Ara h 3 was challenged with peanut and developed grade II anaphylaxis. In serum collected at the

time of challenge a doubling of IgE to the peanut allergen extract was observed compared to allergy testing 9 months earlier. In contrast, IgE levels to Ara h 1, Ara h 2, Ara h 3 and to Ara h 8 were rather unchanged. After another 2 months, Ara h 6 was analyzed and revealed a level of 24 kU_A/l whilst Ara h 2 was 0.12 kU_A/l. We suggest that IgE sensitization to Ara h 6 caused the reaction and conclude that analyses of IgE levels to peanut and peanut components should be performed in connection with a challenge. Furthermore, levels to Ara h 2 below 0.35 kU_A/l may still indicate a risk of severe reaction at the time of challenge since in rare cases, Ara h 6 IgE antibodies may be present without occurrence of IgE antibodies to Ara h 2.

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Introduction

Molecular allergology and component-resolved diagnostics combined with a thorough clinical evaluation have provided improved insight into the allergens causative of clinically relevant sensitization, in particular to allergens of plant origin. Peanut contains several characterized allergen components, such as storage proteins, non-specific lipid transfer protein (nsLTP), a PR-10 protein homologous to the major birch pollen allergen, Bet v 1, and the pan-allergen profilin.

The major peanut allergen Ara h 2 (2S albumin, conglutin), as well as Ara h 1 (7S globulin, vicilin-like protein) and Ara h 3 (11S globulin, glycinin-like protein) are all storage proteins and suggested to be markers of primary sensitization to peanut in individuals who have reacted systemically [1–4]. Ara h 2 is considered to be relatively stable to heat and gastric denaturation [5], and is regarded as the clinically most relevant peanut allergen. The PR-10 allergen Ara h 8 is a labile and low-abundant protein frequently causing mild and local reactions to peanut in regions where birch pollen allergy is common [6, 7] whereas Ara h 9 (nsLTP) is stable to heat and gastric digestion, potentially causing more severe reactions. Ara h 9 appears to be a less relevant allergen in central and northern Europe than in southern Europe [4, 8–10].

Ara h 1, Ara h 2 and Ara h 3, as well as Ara h 8 and Ara h 9 are now available for routine serological testing.

A variant of peanut 2S albumin, designated Ara h 6, has recently been described and found to be a relevant allergen in peanut allergy [3, 11–15]. Since Ara h 2 and Ara h 6 belong to the same protein family, are structurally similar and extensively cross-reactive, it is uncertain to what extent Ara h 6 plays a role in allergy to peanut, distinct from that of Ara h 2, and if evaluation of IgE to Ara h 6 would offer diagnostic value additional to that of Ara h 2.

We wanted to test the clinical relevance of sensitization (≥ 0.35 kU_A/l) to Ara h 8 in the absence of sensitization to Ara h 1, h 2, and h 3 (<0.35 kU_A/l) in children and adolescents. According to reported findings in regard to peanut allergy [6, 7] and consistent with the clinical manifestation of PR-10 allergen sensitization in several other food allergies [16–18], reactions other than OAS would not be likely in such patients. A pre-study was set up in order to evaluate a three-step oral challenge procedure (0.1 + 1 + 10 g of roasted peanuts at 20-min interval) in these patients, which would be less time consuming as compared to our standard procedure [19]. Since we were mainly aiming to exclude the possibility of any systemic reaction in birch pollen-allergic and peanut-sensitized

patients, the challenges were performed in an open fashion. This new procedure of challenge was approved by the Regional Ethical Review Board in Stockholm.

Measurements of IgE antibodies were performed at the Department of Clinical Immunology, Karolinska Hospital (Stockholm, Sweden), and at the R&D Laboratory of Phadia (Uppsala, Sweden), in the case of rAra h 6 using an experimental ImmunoCAP test. Ara h 6 (sequence Acc. No. Q647G9) was produced in *Escherichia coli* as a hexahistidine-tagged recombinant protein using a synthetic gene construct and purified by immobilized metal ion affinity chromatography followed by ion exchange chromatography as described [20]. Experimental ImmunoCAP tests were prepared as described [21].

Case Report

Here we report a case of grade II anaphylaxis in a 15-year-old boy. Four other subjects with the same pattern of peanut sensitization, i.e. IgE to Ara h 8 but not to Ara h 1, Ara h 2 or Ara h 3, did not react at all in the three-step oral peanut challenge.

From early childhood, this patient was allergic to pollen and furred pets (table 1) with symptoms of rhino-conjunctivitis, a medical diagnosis of asthma and atopic eczema. Following a positive allergy test before school age, he has refrained from consumption of tree nuts and peanuts, but has not systematically avoided food items labelled 'may contain' of these ingredients. He had no history of anaphylaxis. He was IgE-tested 9 months prior to challenge and fulfilled our inclusion criteria for the pre-study, i.e. he exhibited IgE antibody levels to Ara h 1, Ara h 2 and Ara h 3 <0.35 kU_A/l and to Ara h 8 ≥ 0.35 kU_A/l (table 2).

At the time of the open challenge, shortly after both the first and second dose of 0.1 and 1 g peanut, respectively, he noticed oral itch which spontaneously disappeared. Shortly after the third dose of 10 g peanut, he complained of stomach pain. Oral antihistamine 10 mg desloratadine was administered without clinical improvement. Then he vomited, had diarrhoea and became increasingly anxious and developed lower respiratory obstruction, but without reduction in SaO₂ (99%). Blood pressure and heart rate were stable and normal. He was given 0.5 mg adrenalin intramuscularly, intravenous fluid and salbutamol inhalation, and improved initially, but again complained of stomach ache, fatigue and was restless. Another dose of 0.5 mg of adrenaline was given intramuscularly and betametason, 4 mg, was administered intravenously.

IgE testing of a blood sample taken at the time of the challenge showed a doubling of IgE antibody concentration to peanut as compared to a sample drawn 9 months earlier. In addition, barely detectable levels of IgE antibody to Ara h 2 and Ara h 9 were noted (table 2); however, not at all accounting for the rise in IgE to the peanut allergen extract. This observation led us to perform an analysis of IgE to Ara h 6 in a blood sample drawn 2 months after the peanut challenge. In contrast to previous observations of a strong correlation between the levels of IgE antibody to Ara h 2 and Ara h 6, this analysis revealed massive sensitization to Ara h 6 (24 kU_A/l, table 2), despite a very modest level of IgE to Ara h 2.

Table 1. Clinical characteristics of a boy with anaphylaxis upon challenge to peanut in September 2010

	2002	2010
Asthma	+	+
Rhinoconjunctivitis	+	+
Eczema	+	+
IgE (kU _A /l) to		
Birch	18	50
Timothy	7	n.d.
Mugwort	4.7	11
Cat	3.0	n.d.
Dog	2.5	n.d.
Horse	1.5	n.d.
Dust mite	n.d.	2.4

n.d. = Not determined; + = symptom present.

Table 2. IgE antibodies to birch pollen, peanut and hazelnut of a boy with anaphylaxis upon challenge to peanut in September 2010

IgE, kU/l	2002	Dec 2009	Sept 2010	Nov 2010
Birch	18	43	n.d.	56
Bet v 1	n.d.	n.d.	n.d.	30
Peanut	7.9	6.3	12	18
Ara h 1	n.d.	<0.10	<0.10	0.06
Ara h 2	n.d.	<0.10	0.22	0.12
Ara h 3	n.d.	<0.10	<0.10	0.10
Ara h 6	n.d.	n.d.	n.d.	24
Ara h 8	n.d.	17	15	16
Ara h 9	n.d.	n.d.	0.21	0.24

n.d. = Not determined.

Discussion

Taken together with previously published findings regarding the clinical importance of IgE sensitization to peanut storage proteins and the milder nature of Ara h 8-mediated reactions to peanut, we find it likely that the anaphylaxis in this boy at the peanut challenge described was mainly caused by IgE to Ara h 6 and not to Ara h 2. While Ara h 2 and Ara h 6 exhibit an intermediate level of overall sequence identity (53–57%), cases of isolated sensitization to Ara h 6 are rare as the two allergens share several, even if not all important IgE binding epitopes, and have been found to cross-react extensively [14].

Lessons learned from this case include the following:

- (1) in rare cases, IgE to Ara h 6 may occur in the absence of sensitization to Ara h 2 and cause a severe reaction to peanut.
- (2) As significant changes in sensitization pattern may occur over time, an oral food challenge should be preceded by a newly performed IgE analysis. If IgE to peanut has increased whilst IgE to Ara h 8 remained stable, induction of primary sensitization to peanut may have taken place, increasing the risk of a severe reaction.
- (3) In the absence of a routine test for IgE to Ara h 6, the closely related Ara h 2 may serve as a marker of sensitization both to itself and to Ara h 6. Even very low levels (0.1–0.35 kU_A/l) of IgE to Ara h 2 could be regarded as a warning sign of a possible reaction upon oral exposure.

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