Ara h 1-digesta lose sensitizing activity when separated into fractions

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Introduction

The peanut allergen Ara h 1 is a major allergen. We have shown in a previous study that Ara h 1 retains both its sensitising and eliciting capacity when digested.

The aim of this study was to investigate the allergenic capacity of fractions of such digested Ara h 1 in a Brown Norway (BN) rat model.

Methods

Groups of BN rats were immunised i.p. three times with use of adjuvant with 200 µg of either PBS (control), intact Ara h 1, whole pool of Ara h 1 digesta, or two different fractions of the digested Ara h 1.

Digested Ara h 1 was analysed for residual intact Ara h 1 by RP-HPLC, aggregation profiles by gel permeation chromatography (GPC) and peptide masses by MALDI-TOF mass spectrometry (MS).

Sera from BN rats were analysed for specifik IgG1, IgG2a and IgE titres and the avidity of the antibodies were measured in ELISAs.

Results

RP-HPLC showed that no residual intact Ara h 1 was left in the Ara h 1-digesta. MALDI-TOF MS showed that peptides in the pool of Ara h 1-digesta were ≤ 4 kDa, peptides in fraction of large complexes were ≤ 4 kDa, and peptides in fraction of small complexes were ≤ 3 kDa (Fig. 1, left column).

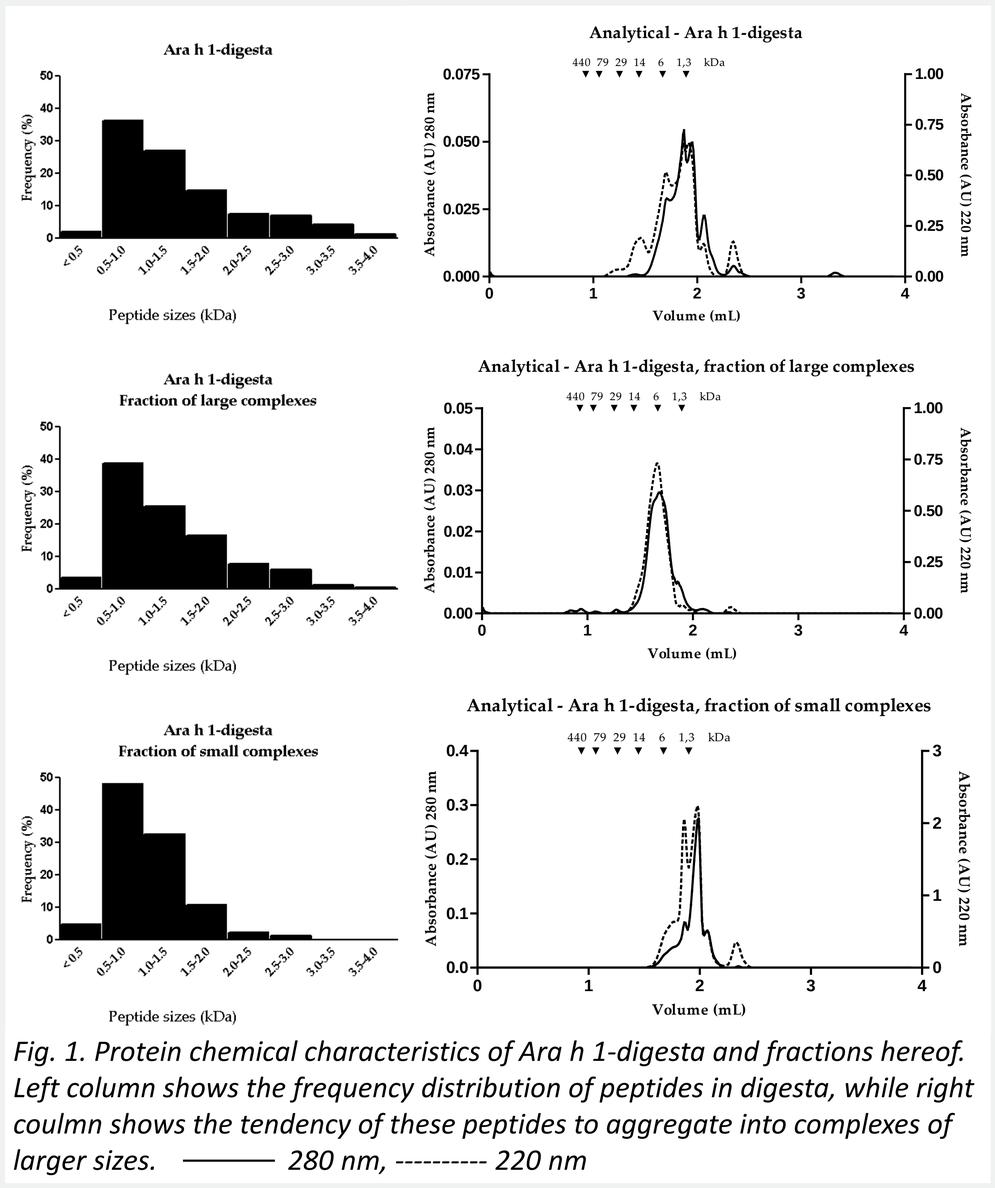

Fig. 1. Protein chemical characteristics of Ara h 1-digesta and fractions hereof. Left column shows the frequency distribution of peptides in digesta, while right coulmn shows the tendency of these peptides to aggregate into complexes of larger sizes. ——— 280 nm, ---------- 220 nm

GPC analyses showed that the peptides had a tendency to aggregate, though to different degrees (Fig. 1, right column). It was indicated that 25% of peptides in the pool of Ara h 1-digesta were aggregated to complexes of up to M_r 104, 53% of peptides in fraction of large complexes were aggregated to complexes of up to M_r 56, and 7% of peptides in fraction of small complexes were aggregated to complexes of up to M_r 9.

The BN rat study showed that while both intact Ara h 1 and the pool of Ara h 1-digesta had sensitising capacity, both fractions of digesta had no sensitising capacity (Fig. 2). However, rats immunised with intact Ara h 1 could still react with both fractions in a significant way.

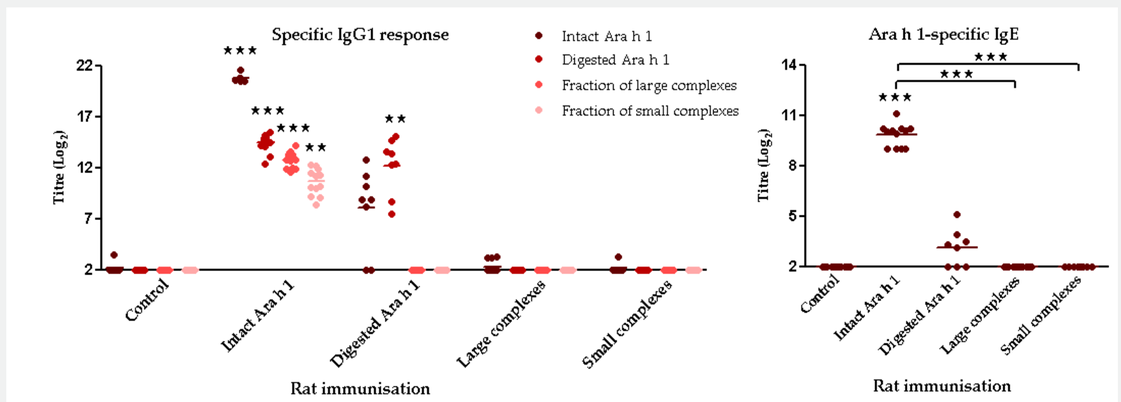

Fig. 2. Specific IgG1 and IgE antibody responses of individual rats.

Results from avidity measurements indicated that antibodies from rats immunised with intact Ara h 1 had a statistically significant higher avidity towards the intact Ara h 1 compared to antibodies from rats immunised with digested Ara h 1 (Fig. 3). Also antibodies from rats immunised with intact Ara h 1 had higher avidity towards the intact Ara h 1 than to the fraction of larges complexes of digested Ara h 1.

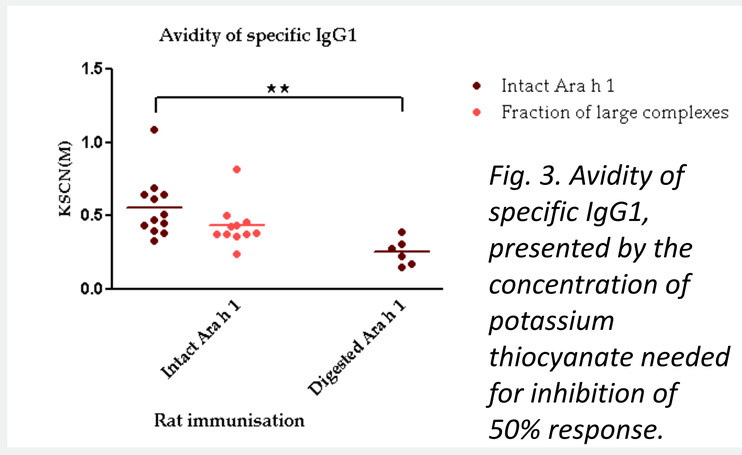

Fig. 3. Avidity of specific IgG1, presented by the concentration of potassium thiocyanate needed for inhibition of 50% response.

Conclusion

This study confirms that even though Ara h 1 is digested to small peptides, it retains the sensitising capacity.

However, when digested Ara h 1 was separated into fractions the sensitising capacity was lost. A possible explanation for this could be that the stability of the Ara h 1-digesta solution is lost when separated into fractions, or that most peptides need to be present to serve as adjuvant for each other augmenting the immune response against other peptides and therefore needs to be administered together.