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Detection of peanut allergen in human blood after consumption of peanuts is skewed by endogenous immunoglobulins

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ABSTRACT

Some studies have suggested that allergens may appear in the circulation after ingestion of allergenic food sources. The reported levels of allergen in serum, however, are low, and conclusions between studies differ. Here, we investigated factors that determine the detection of allergens in serum after consumption of peanuts. Ten healthy volunteers ingested 100 g of light-roasted peanuts. Serum samples were taken at regular intervals for six hours. A double monoclonal sandwich ELISA was used to analyse the presence and quantity of the major peanut allergen Ara h 6 in serum.

In 4 out of 10 subjects, no Ara h 6 could be detected. Purified Ara h 6 that was digested *in vitro* was still reactive in the ELISA, rejecting the possibility that digestion leads to small peptides that could not be detected. Spiking of purified Ara h 6 in baseline serum showed that the pre-ingestion serum of these four subjects partially prevented Ara h 6 to react in the ELISA, with a reduction of reactivity of up to 3 orders of magnitude or more. Pre-ingestion serum of the other six subjects did not show such an effect. The reduction of reactivity of Ara h 6 coincided with high titres of IgG and IgG4, and removal of IgG from pre-ingestion serum abolished this effect completely, indicating that IgG and IgG4 inhibited the reactivity of Ara h 6 in the ELISA.

We conclude that some individuals have IgG and IgG4 against food allergens in their blood, which interferes with detection of such food allergens in serum. Because this effect does not occur for each individual, the possibility of such interference should be taken into consideration when interpreting immunochemical studies on the absorption of food allergens in serum.

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1. Introduction

Food allergies affect 2–3% of the population in Western countries, and peanut allergy is one of the most important food allergies because of 1) its persistent character, 2) the low amounts of peanut needed to trigger a reaction, and 3) peanut being a widely used food ingredient. Peanut conglutins Ara h 2 and Ara h 6 are the most potent allergens with regard to IgE-binding, skin prick test, and basophil test. IgE to these allergens has a high positive predictive value for diagnosing peanut allergy (Klemans et al., 2014).

A characteristic of conglutins (2S-albumins) is their relative stability to digestion (Astwood et al., 1996; Pfeifer et al., 2015). Digestion of Ara h

2 and Ara h 6 by pepsin and trypsin has been investigated in the past, showing that these are only partially hydrolysed resulting in relatively large peptides of around 4 to 5 kDa and 10 kDa in size (Sen et al., 2002; Koppelman et al., 2010; Apostolovic et al., 2016). Such digestion-resistant peptides are exposed to the intestinal immune system as large protein fragments, potentially explaining the potent allergenicity of these allergens with regard to their capacity to sensitize. It has also been shown that digestion-resistant peptides can be taken up in the circulation, leading to systemic reactions in peanut allergic individuals. Several studies investigated the uptake of digestion-stable food allergens such as peanut (Dirks et al., 2005; Baumert et al., 2009), egg (Husby et al., 1987) and wheat (Matsuo et al., 2005) in healthy individuals and animal models (Bernard et al., 2014). Ara h 2 and Ara h 6 have also been detected in breast milk of lactating women (Vadas et al., 2001; Schocker et al., 2016) and mice (Bernard et al., 2014). Moreover, a case report revealed that transfusion of a blood product into a peanut allergic recipient led to an anaphylactic reaction explained by residual peanut in

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the blood of one of the donors who consumed peanut prior to donation (Jacobs et al., 2011). Together these reports indicate that immunologically active fragments of peanut allergens can at least partially survive the gastro-intestinal tract, and may appear in the circulation to be transported to various compartments of the body. However, the reported values of peanut allergen taken up are low, and often close to the limit of detection. Also, large variability between studies and between individuals within studies is observed. Considering the digestion resistance of peanut conglutins and their fairly high abundance in peanut, one would expect more consistent and higher levels of such proteins in the circulation if these were indeed taken up by the intestine and transported into the blood.

The aim of this work was to identify possible confounding factors for the detection of peanut allergens in serum after consumption of peanut. We selected Ara h 6 as representative of the potent peanut conglutinin allergens. Ara h 6 is biochemically well characterised and appears as a homogenous protein, with limited isoforms. We recruited a group of 10 healthy individuals, sampled blood before and after digestion of peanuts and analysed their serum for the presence of Ara h 6 at various time points. Moreover, we performed spiking experiments to investigate if detected levels corresponded to expected levels, and which sera-derived factors could be responsible for interference.

2. Materials and Methods

2.1. Healthy volunteers / subject

Ten healthy subjects were recruited via social media and email database of the university's human nutrition division (Wageningen University, the Netherlands). Exclusion criteria were having (suspected) allergic symptoms after peanut ingestion, smoking, use of illicit drugs, NSAIDs on a chronic basis, or use of any medication for gastric or intestinal complaints. None of the subjects donated blood during the last six weeks before the start of the study. Subjects were also instructed not to eat peanut-containing foods nor use alcohol for 2 days prior to the test day.

This study was approved by the medical ethical committee of Wageningen University (METC-WU 15/34; NTR5655), and conducted in accordance with the Declaration of Helsinki (revised version, October 2008, Seoul). All subjects gave their written informed consent after the nature and possible consequences of the study had been fully explained.

Each subject came to the university while having fasted overnight. Pre-ingestion blood was sampled after which they were asked to consume 100 g of light-roasted, unsalted peanuts obtained from a local supermarket (Albert Heijn; private label product) within 10 min. Blood was sampled in serum separator tubes 30, 60, 120, 240, and 360 min after finishing peanut consumption. Blood was left to clot in the dark for at least 20 min at room temperature, after which the tubes were centrifuged at 2000 g for 10 min at room temperature to obtain serum, which was directly aliquoted and stored at -80°C until further analysis.

2.2. Peanut allergens

Virginia peanuts obtained from the US were used to purify the various Ara h 6 preparations. Ara h 6 was purified essentially as described earlier (Koppelman et al., 2005) with one additional hydrophobic interaction chromatography step to remove traces of impurities. Post-translationally proteolytically processed Ara h 6 (hereafter named naturally processed Ara h 6) was purified from side fractions of the Ara h 6 purification (Koppelman S.J., unpublished data), which contained disulfide-linked molecules consisting of a 5 and 10 kDa chain with estimated purity of >95%. Identity was proven by peptide mass finger printing (data not shown). Digestion-resistant peptides from peanut conglutinin (equimolar mix of the heavy isoform of Ara h 2, the light isoform of Ara h 2, and Ara h 6) were prepared by incubation of peanut conglutinin

with immobilized trypsin (Apostolovic et al., 2016). The fraction of Ara h 6 in the equimolar mix was 0.30 based on mass. The protein concentration of the digestion-resistant peptide mix (hereafter named digested mix of Ara h 2 and Ara h 6) is expressed in mg of Ara h 6 per mL to simplify comparison with other Ara h 6 preparations.

2.3. ELISAs

The Ara h 6 ELISA was from Indoor Biotechnologies (EL-AH 6, Cardiff, UK), utilizing two monoclonal antibodies. The instructions of the manufacturer were followed, except that in some cases the reference material was replaced by other Ara h 6 preparations. By using extra dilutions of the standard, the lower limit of quantification was determined to be 0.024 ng/mL (defined as the value corresponding to an absorbance of the blank + 10 SD), and the lower limit of detection to be 0.012 (defined as the value corresponding to an absorbance of the blank + 3 SD). Samples were tested in duplicate and experiments were performed at least twice. To compare reactivity of different Ara h 6 forms, EC_{50} values, defined as the concentration that leads to half-maximal absorbance, were calculated using the Graphpad Prism software, version 6.07.

ELISAs for IgG and IgG4 were set-up as follows. Purified Ara h 6 was coated on ELISA plates (Nunc MaxiSorp® flat-bottom 96 well plate) at 3 $\mu\text{g/mL}$ in PBS (pH 7.4; 136.89 mM NaCl, 1.47 mM KH_2PO_4 , 8.10 mM Na_2HPO_4 , 2.68 mM KCl), 100 $\mu\text{L/well}$, overnight at 4°C . Plates were washed once with PBS and then blocked with PBS containing 2% (W/W) bovine serum albumin (BSA, A6003, Sigma Aldrich, Zwijndrecht, NL), for 2 h at 37°C under shaking conditions (300 rpm). After washing (3 times with PBS containing 0.05% Tween 20 (PBS-T)), diluted serum samples were pipetted in the wells in duplicate and were allowed to incubate for 2 h at 37°C under shaking conditions (300 rpm). Wells were washed again (3 times with PBS-T) and a 1:500 dilution of horseradish peroxidase conjugated anti-human IgG (05-4220, Invitrogen, Breda, NL) or 1:500 dilution of anti-human IgG4 (ab99817, Abcam, Cambridge, UK) in PBS-T with 1% BSA was applied on the plates for 1.5 h at 37°C under shaking conditions (300 rpm). Plates were further developed using 100 μL of TMB substrate (T0440, Sigma Aldrich). Colour development was stopped after 10 min by adding 100 μL of Stop reagent (S5814, Sigma Aldrich). Absorbances with oversaturated values were imputed at 4.1, representing the highest absorbance that can be measured with the ELISA plate reader (Synergy HT, BioTek, Winooski, VT, USA).

2.4. Removal of IgG from serum

Two times diluted pre-digestion serum (in PBS) was applied to a Protein G Sepharose Column (6518, BioVision, ITC Diagnostics bv, Uithoorn, NL), equilibrated with PBS. The flow-through was collected and re-applied to the column four times. The final flow-through was collected as IgG-depleted serum. The column was washed and re-equilibrated according to the instructions of the manufacturer.

3. Results and Discussion

We applied an ELISA specific for Ara h 6 detection to study the presence of this allergen in serum of healthy individuals after consuming peanuts. Several hypotheses were tested to explain why the detected levels of Ara h 6 are low and variable.

3.1. Hypotheses formulation

An overview of some relevant subject characteristics of the study is provided in Table 1. Upon ingestion of peanuts, the serum samples at $t = 60$ min were tested for the presence and quantity of Ara h 6. In serum of four subjects (out of ten), Ara h 6 was undetectable, while in the serum of the other six subjects low levels of Ara h 6 of 0.30 ± 0.16 ng/mL could be detected. The total amount of peanuts consumed was 100 g. Using a protein content of 25% (Oerise et al., 1974), and

Table 1
Subject characteristics and Ara h 6 levels.

ID	M/F	Age	Consumption products high peanut content ¹	Consumption products low peanut content ²	[Ara h 6] at 60 min (ng/mL)
1	M	21	Almost daily	1–3 × per month	ND
2	F	20	2–5 × per week	1–3 × per month	0.38
3	F	22	1–3 × per month	2–5 × per week	0.09
4	M	21	1–3 × per month	1 × per week	0.15
5	F	21	2–5 × per week	1–3 × per month	0.26
6	F	22	2–5 × per week	<1 × per month	0.36
7	F	22	1–3 × per month	2–5 × per week	0.54
8	M	23	1 × per week	1–3 × per month	ND
9	M	21	Almost daily	1–3 × per month	ND
10	F	23	1–3 × per month	1–3 × per month	ND

M/F = Male/Female.

ND = Not detected.

¹ Examples: peanuts/peanut butter/ peanut sauce.

² Examples: candy bars/ cookies/ muesli (bars).

taking into account that the Ara h 6 content of (Virginia) peanuts is approximately 6% (Koppelman et al., 2016), the subjects ingested approximately 1.5 g of Ara h 6. If all had been taken up in the circulation, assuming no turnover of Ara h 6 and a blood volume of 5 L, this would have led to a concentration of 0.3 g/L, corresponding to 300,000 ng/mL. It is expected that the intestine functions as a barrier, preventing the uptake of larger undigested dietary components. The observation, however, that only 0.3 ng/mL is found, and not even in serum from all subjects, possibly illustrates a minor and variable part of ingested Ara h 6 being detected in serum. Three hypotheses were formulated to explain the low levels of uptake of Ara h 6 in serum. In these hypotheses we assume that there are additional factors beyond intestinal uptake function: 1) the ELISA is not reactive for hydrolysed or digested Ara h 6; 2) a matrix effect disturbs the analytical procedure; or 3) the time point chosen at first for serum collection of 60 min is not optimal. The sections below provide the results of testing the hypotheses.

3.2. Reactivity of hydrolysed or digested Ara h 6 in the Ara h 6 ELISA

The epitopes that are recognised by the monoclonal antibodies utilised in the Ara h 6 ELISA are, to our knowledge, not mapped (personal communication with ELISA manufacturer, dd. March 2016). The ELISA manufacturer also does not provide information on the possible reactivity of processed or digested Ara h 6. It is therefore not known whether this ELISA can detect digested Ara h 6. A naturally hydrolysed (processed) form of Ara h 6 occurs naturally in peanut (Bernard et al., 2007) and was recently purified and characterized biochemically (Koppelman S.J., unpublished data). This post-translationally proteolytically processed form is composed of an N-terminal part of about 5 kDa linked by disulphide bonds to the C-terminal part of about 10 kDa, highly similar to the profile for digested Ara h 2 and Ara h 6 (Apostolovic et al., 2016), and was used to investigate the detection of digested Ara h 6 by the ELISA. The reactivity of the three different Ara h 6 preparations was tested in the Ara h 6 ELISA. Fig. 1 shows the calibration curve of the purified (intact) Ara h 6, a curve of the naturally processed form of Ara h 6, and a curve of a mix of Ara h 2 and Ara h 6 treated with trypsin (normalised for Ara h 6 concentration). Intact and naturally processed Ara h 6 show a comparable reactivity illustrated by EC₅₀ values (concentration that leads to half-maximal absorbance) of 2.6 and 3.0 ng/mL, respectively. To mimic digestion under more physiological conditions, a mix of Ara h 2 and Ara h 6 was treated with trypsin, leading to a protein profile for Ara h 6 consisting of a 5 kDa and 10 kDa fragment held together by disulphide bridges as described above (Apostolovic et al., 2016). These digestion-resistant peptides were also tested in the Ara h 6 ELISA, and show a curve overlapping with that of naturally processed Ara h 6 (EC₅₀ is 2.8 ng/mL). Together, this strongly suggests that Ara h 6

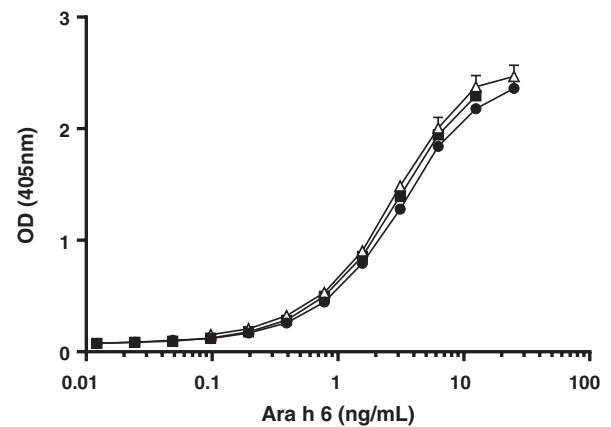


Fig. 1. Reactivity of Ara h 6 and hydrolysed forms of Ara h 6 in the Ara h 6 ELISA. Open triangles (Δ): Purified native (intact) Ara h 6; Closed circles (●): naturally processed Ara h 6 as purified from peanuts; Closed squares (■): mix of Ara h 2 and Ara h 6 treated with trypsin (Ara h 6 concentration in the mix was calculated and used on X-axis (as described in Methods) to enable comparison with other Ara h 6 forms).

digested *in vivo* is still reactive in the Ara h 6 ELISA, although perhaps at a slightly lower level. It is therefore unlikely that digestion of Ara h 6 prevents the detection by ELISA. The processed form of Ara h 6 purified from peanut was used as standard for the following ELISA experiments. The dynamic range of the calibration curve is 3 orders of magnitude, which allows the detection of large differences in reactivity of samples.

3.3. Matrix effects of serum

A serum sample was obtained from all subjects before eating peanuts. This pre-ingestion serum was spiked with various concentrations of naturally processed Ara h 6 to investigate any possible matrix effect. Fig. 2 shows that in the majority of sera Ara h 6 could be detected comparable to Ara h 6 in buffer. However, a marked inhibition of reactivity is observed in sera from four subjects. For subject no. 10, this inhibition is pronounced for low concentrations of Ara h 6, while detection was good for higher concentrations. For subjects no. 1, 8, and 9, the inhibition was much stronger, and for subject no. 9 the detection of even the highest concentration of Ara h 6 (12.5 ng/mL) was still very low. Taking into account the LOD of the ELISA, i.e. 0.012 ng/mL, this inhibitory effect is at least 3 orders of magnitude.

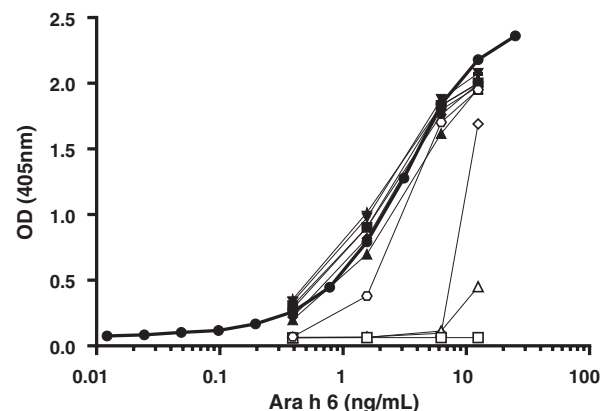


Fig. 2. Reactivity of naturally processed Ara h 6 when mixed into pre-ingestion serum for the study subjects. Calibration curve (closed circles: ●) is shown for the full dilution range. For spiking the baseline sera 0.4 ng/mL, 1.6 ng/mL, 6.3 ng/mL, and 12.5 ng/mL of naturally processed Ara h 6 was used. Subject no. 1: ◇; subject no. 2: ▼; subject no. 3: ▲; subject no. 4: ■; subject no. 5: ●; subject no. 6: ◆; subject no. 7: ✱; subject no. 8: Δ; subject no. 9: □; subject no. 10: ○.

We hypothesized that competing antibodies present in serum may cause the observed inhibitory effect. Immunoglobulin (Ig) toward dietary proteins is common and IgG may increase as a result of frequent ingestion of immune-reactive dietary proteins (Husby, 2000; Tay et al., 2007). A strong increase in IgG antibodies against food proteins such as peanut and egg is seen in immunotherapy studies. Oral immunotherapy with allergic subjects that normally avoid the offending food often results in an increase of serum IgG and functional blocking activity is demonstrated in basophil histamine release assays (Strait et al., 2006; Buchanan et al., 2007; Jones et al., 2009; Vickery et al., 2013). However, our subjects do not avoid but are regularly exposed to peanut (Table 1), and it is possible that they have IgG to peanut in their circulation. We have tested the pre-ingestion sera of the subjects in a direct ELISA for Ara h 6-specific IgG (Fig. 3A) and Ara h 6-specific IgG4 (Fig. 3B). The sera that did not show inhibitory effects in the Ara h 6 ELISA had low or absent IgG reactivity. In contrast, the sera from subject no. 1, 8, and 9 had considerable IgG reactivity for Ara h 6. The IgG titres coincided with the inhibitory potency. Interestingly, this picture is even more pronounced for antibodies of the IgG4 subclass directed to Ara h 6 (Fig. 3B). Serum from subject no. 8 and 9 could be diluted over 1000 fold before losing reactivity. The sera that did not show an inhibitory effect in the Ara h 6 ELISA did not show clear IgG4 reactivity. Further evidence that IgG/IgG4 mediate the inhibitory effect is obtained when IgG was removed from serum using Protein G Sepharose. Fig. 3C shows that IgG and IgG4 was removed efficiently (using the sera with highest titres; i.e. from subjects no. 8 and no. 9) and this resulted in almost complete abolishment of the inhibitory effect on Ara h 6 detection (Fig. 3D). While in untreated serum from subject no. 8 and no. 9 a serum-dilution dependent effect on detection is observed, the IgG-depleted sera

allowed detection and quantification of the Ara h 6 spikes well ($> 80\%$ at a serum dilution of 4-fold; Fig. 3D).

A large inhibitory capacity of the serum coincided with a high titre of Ara h 6-specific IgG and IgG4. High IgG and IgG4 levels are seen especially in subjects with frequent habitual peanut ingestion, suggesting a possible association. For both characteristics of the sera the order of the effect was: subject no.9 $>$ no.8 $>$ no.1 $>$ no.10. Moreover, the sera from subjects that showed no inhibitory effect also had low or non-detectable Ara h 6-specific IgG or IgG4 titres. Thus, we conclude that for part of the subjects there is an inhibitory effect mediated by IgG and/or IgG4 against Ara h 6 that interferes with and skews the quantification of Ara h 6 by ELISA. Earlier studies reported interaction of food proteins with immunoglobulins, in particular observed in patients with Coeliac disease (Mohammed et al., 1976; Husby et al., 1986). However, a potential inhibiting effect of such interaction on the detection of food allergens by immunochemical methods has not been considered so far.

3.4. Time course of appearance of Ara h 6 in serum

Due to the inhibitory effects of serum of subject no. 1, 8, 9, and 10 it was not possible to assess the amount of Ara h 6 that enters the circulation of these subjects and consequently non-detectable levels were reported in Table 1. Because the inhibitory effect is dependent on the Ara h 6 concentration (Fig. 2) and because the optimal time point for determining the presence of Ara h 6 in serum is not yet known, we have evaluated the time course of appearance of Ara h 6 in serum for all 10 subjects from 30 min up to six hours, speculating that at some point in time the Ara h 6 would be high enough to overcome the inhibitory

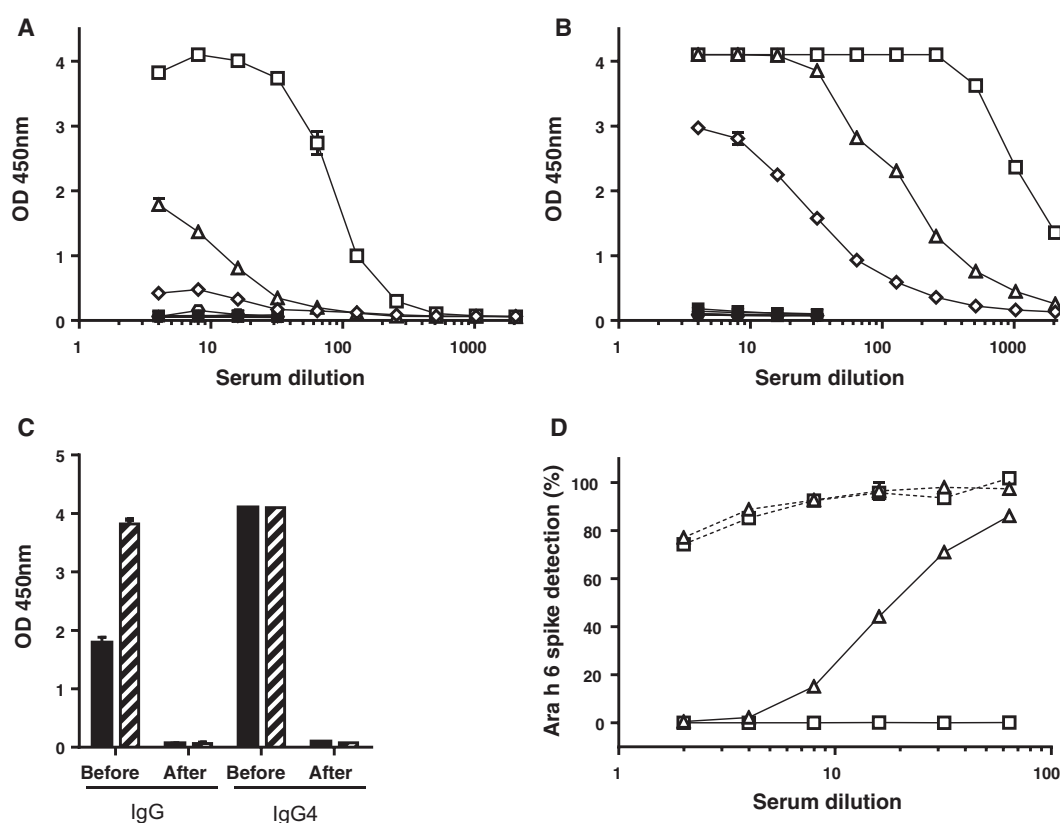


Fig. 3. IgG and IgG4 specific for naturally processed Ara h 6 in serum of study subjects inhibits reactivity in the Ara h 6 ELISA. IgG and IgG4 ELISAs were performed to compare the Ara h 6-specific IgG and IgG4 titers of the sera from the study subjects. Markers are the same as in Fig. 2. Panel A: Ara h 6-IgG titers of all subjects. Panel B: Ara h 6-specific IgG4 titers for all subjects. Panel C: Ara h 6-specific IgG and IgG4 in serum from subjects no. 8 (black bar) and 9 (Striped bar) (subjects with highest inhibitory potency) before and after cleanup with Protein G Sepharose. Panel D: Detection of different levels of naturally processed Ara h 6 in serum from subject no. 8 and 9 (subjects with lowest detection of naturally processed Ara h 6 spike) before and after cleanup with Protein G Sepharose. Solid line: before cleanup; dotted line: after cleanup.

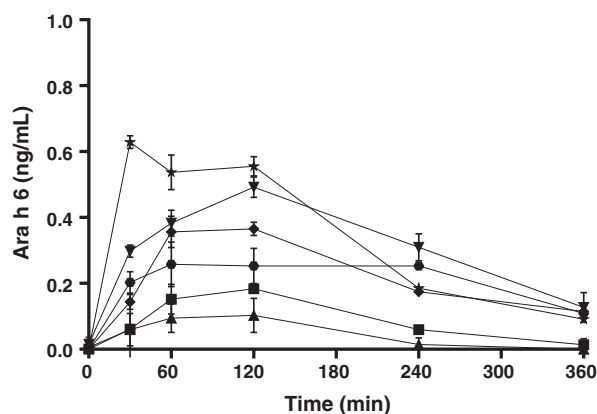


Fig. 4. Naturally processed Ara h 6 detection in serum of study subjects after peanut ingestion. Ara h 6 ELISAs were performed to analyze the level of naturally processed Ara h 6 over time following 100 g of peanut intake. The values are shown as the average of two independent ELISAs. Markers are the same as in Fig. 2. The AUC over time is on average 74.2 ± 42.1 ng·min/mL.

effect. Fig. 4 shows that only for the subjects whose serum did not inhibit the Ara h 6 ELISA, Ara h 6 could be detected at any time point.

Two key observations can be made from Fig. 4. First, the detected levels of Ara h 6 seem to be fairly low. Considering the dose of Ara h 6 ingested with 100 g of peanuts (appr. 1.5 g), an average peak level of 0.34 ng/mL (± 0.20 ng/mL) after 60 min reflects only about 0.0001% of the intake at that moment in time. This low peak level in serum could be due to low uptake or to rapid clearance, or a combination of both. It is consistent with the view that the intestine functions as a barrier against the uptake of oligopeptides (Miner-Williams et al., 2014).

Second, the time point where the maximal concentration of Ara h 6 is found differs per subject. Because maximum levels are detected in serum after 120 min in most subjects this seems a suitable sampling time point for uptake detection, although a time course should be made for full quantification. Because all subjects have lower than peak levels after 4 h, a period up to 4–6 h after ingestion presents a good time-frame to capture the progress of allergen appearance in serum. This is in line with other reports on the appearance of food allergens in circulation upon ingestion (Husby et al., 1986; Dirks et al., 2005; Baumert et al., 2009).

3.5. Implications for future research

Although literature describes some variation in peak levels of food allergens in the circulation after ingestion of allergen-containing foods, the levels are most often in the ng/mL range. As we demonstrate here, such low levels may be undetectable by immunochemical techniques in cases where the subjects have IgG/IgG4 against the relevant protein of interest. We utilise a sandwich ELISA, a common technique to detect and quantify allergens in complex matrices. A sandwich ELISA requires binding of at least two antibodies to two different epitopes of the allergen. Therefore, binding of an allergen to endogenous IgG/IgG4 may prevent reactivity in sandwich ELISA due to steric hindrance or occupation of amino acid sequences at or in close proximity to the antibody epitopes. ELISA formats that require only one antibody-analyte interaction (e.g. inhibition ELISA, direct ELISA, RAST inhibition (Hamilton, 2014)), may mathematically have a lower change for inhibition. The sensitivity of such formats is, however, not as good as that of sandwich ELISA, and possibly not sufficient to detect and quantify allergens in the ng/mL range. A very sensitive approach is the basophil histamine release assay. Dirks et al., (2005) studied peanut allergen uptake using the basophil histamine release assay. While this was not designed as a quantitative assay, a dose-response effect was seen, and the time of peak presence of peanut protein was between 15 min and 2 h. Using the sensitivity of the assay (determined to be

5 pg/mL) the estimated concentration of peanut protein was in the higher pg/mL range, which is comparable to our observations. The basophil histamine release assay depends on allergen-antibody interaction, so the presence of IgG/IgG4 in the serum of the subjects could disturb this assay as well. Because samples of 10 subjects were pooled in this study, it is not clear if individual inhibitory effects could have occurred.

Assays independent of analyte-antibody interaction may provide a way to circumvent such inhibitory effect. Mass spectrometry has been applied to detect cow's milk proteins (Coscia et al., 2012) and peanut protein (Schocker et al., 2016) in human breast milk, however the applied technique (i.e. peptide mass fingerprinting) is not quantitative. Truly quantitative mass spectrometry assays have been developed for peanut allergens, however these have not yet been applied for quantification of low concentrations of peanut allergens in serum or breast milk (Johnson et al., 2016).

When comparing the concentration ranges reported for food allergens in serum with those of food allergens in breast milk, it seems that the concentrations in breast milk are higher. It can be hypothesized that peanut protein is transported via the circulation at low concentrations and is potentially circulated through the lymphatic system and excreted by the mammary gland, building up higher concentrations in breast milk. The role of IgG/IgG4 in this process is unknown. Bernard et al. (2014) showed in a mouse model that Ara h 6, once in breast milk, could also interact with IgG. Whether this is also the case in humans needs to be further investigated. Also, the mechanism of the binding of IgG/IgG4 in relation to the transportation and clearance of food allergens in the circulation needs to be further investigated in order to better understand the relevance of intact food allergens (or large immunoreactive peptides of these allergens) circulating in serum after consumption.

3.6. Conclusions

We conclude that the presence of Ara h 6-specific IgG and IgG4 antibodies is a determining factor in the detection of Ara h 6 in serum. These antibodies, which are found in the blood circulation of healthy individuals and may be induced by consumption of peanut, interfere with the detection and quantification of Ara h 6 in serum. This may be a broader phenomenon in studies on the uptake of food allergens in circulation, and may explain why variable levels of food allergen in serum have been reported in literature.

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Conflict of interest

The authors declare that they have no conflict of interest. For the avoidance of doubt, SJK reports to be consultant of DBV Technologies, a company involved in development of epicutaneous immunotherapy for food allergies, which is outside the scope of the research presented here.

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