

Immunochemical characterisation of structure and allergenicity of peanut 2S albumins using different formats of immunoassays

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Abstract Proteins of the 2S albumin family, such as Ara h2 and Ara h6, are most frequently involved in peanut allergy. We have developed a reverse enzyme allergosorbent test (EAST) in which total serum IgE antibodies are first captured by immobilised anti-human IgE monoclonal antibodies, and then the binding of the anti-Ara h2 and anti-Ara h6 specific IgE to the corresponding labelled allergens is measured. This reverse immunoassay was used either as a direct EAST or as an EAST inhibition assay to study the interactions of whole peanut protein extract and purified Ara h2 and Ara h6 with IgE antibodies from peanut-allergic patients. Finally, we identified some IgE-binding epitopes on Ara h6 using a format of EAST in which the protein is immobilised in a particular, well defined, manner through interactions with specific monoclonal antibodies (mAbs) coated on the micro-plates. The fine specificity of those mAbs has been characterised at the epitope level, and their binding to the allergen thus masks a known particular epitope and makes it unavailable for recognition by IgE antibodies. The reverse EAST increased the ratio specific signal/background. It avoids interferences with competitors such as anti-peanut protein IgG antibodies and allows the study of the specificity and/or affinity of the interactions between IgE antibodies and Ara h2 or Ara h6 with a higher sensitivity and accuracy than the conventional EAST. The

EAST results obtained when the allergens are presented by specific mAbs suggest that the homologous molecular domain(s) in peanut 2S albumins encompass major IgE epitope(s) and are strongly involved in peanut allergenicity.

Keywords Allergy · Peanut 2S albumins · Specific IgE antibodies · Reverse immunoassay · Immuno-cross-reactivity

Introduction

About 1% of children under 5 years of age are allergic to peanut [1]. Severe reactions such as Quinck Oedema and anaphylactic shock are observed after peanut ingestion. Most of those clinical manifestations are caused by IgE-mediated hypersensitivity reactions. The study of interactions between IgE antibodies and allergens is widely used. It is noteworthy that the same kind of tests may be used for two different purposes:

One is to detect or confirm the diagnostic of a specific allergy/sensitisation in a patient provided that a standard well characterised allergen is used as reference material and the other one is to assess the allergenicity/IgE-binding capacity of a compound, which is related to a known allergen using the serum of relevant and well-characterised patients whose allergy to the source (food or protein) is confirmed. It is then a useful tool to study the allergenic structures of proteins and peptides and more generally to study the relationship between structure and allergenicity.

Numerous methodologies are performed to detect and measure specific IgE antibodies and to study their interactions with allergens. Since the development of the original radio allergosorbent test (RAST) in the 1970s

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[2, 3], different kinds of tests have been developed. In order to increase the sensitivity of the detection of specific IgE antibodies, anti-IgE antibodies were labelled with enzymes or fluorescent markers. In a previous study, we developed an enzyme allergeo-sorbent test (EAST) in which specific anti-peanut IgE antibodies were detected using an anti-IgE monoclonal antibody labelled to acetylcholine esterase (AChE), giving a limit of detection (LoD) below 0.1 IU/mL. Predictive values, i.e. specificity and sensitivity, were calculated showing that an anti-peanut protein IgE response above 5 IU/mL was associated with a 95% risk of being allergic [4]. However, some false-negative results were observed in patients whose peanut allergy was clinically confirmed but in whom no specific IgE antibodies were detected. Actually, in most of the methods using immobilised allergens such as conventional RAST and EAST, different events such as interactions with specific IgG antibodies may impair the binding to IgE antibodies. To avoid such competition, a reverse immunoassay involving the immobilisation of an anti-IgE on the solid phase and the use of a biotinylated allergen as tracer was proposed [5, 6]. We developed such reverse immunoassays that we used either as a direct or an inhibition test to analyse the allergenicity, i.e. the IgE-binding capacity, of peanut proteins during thermal treatments. This assay proved able to provide the quantification of IC_{50} values (i.e. concentration of competitor that inhibits 50% of the IgE binding to the native allergen) of processed Ara h1 and Ara h2 with a good sensitivity and specificity [7].

The structural and functional characteristics of the different allergens and their proportion in a complex mixture such as a whole peanut protein extract (WPPE) makes it difficult to standardise the test, in which results may also vary depending on the variety and conditions of storage of peanuts and on the procedures used for protein extraction [8–10]. Peanut 2S albumins are major and potent allergens [11, 12]. Component-resolved diagnostic using purified or recombinant proteins as well as synthetic peptides has been used for peanut allergy diagnosis with a good sensitivity and specificity [13, 14].

The aim of the present study was to develop a strategy using various immunochemical methodologies to improve the determination of the IgE response specific to peanut proteins and the characterisation of major allergens involved in peanut allergy such as 2S albumins, then the identification of IgE-binding epitopes on Ara h6 and, more generally, to assess the impact of structural modifications on the allergenicity of proteins.

We have developed a reverse enzyme immunoassay in which total serum IgE antibodies are captured by anti-human IgE monoclonal antibodies coated on micro-titre plates, and the binding of anti-peanut protein-specific IgE is measured using the corresponding labelled allergens. This

reverse immunoassay was used either as a direct EAST or as an EAST inhibition assay to study the interactions of whole peanut protein extract and purified Ara h2 and Ara h6 with IgE antibodies from peanut-allergic patients.

Last, we identified some IgE-binding epitopes on Ara h6 using a conventional format of EAST but in which the protein is immobilised in a particular well-defined manner through interactions with specific monoclonal antibodies (mAbs) coated on the micro-plates. The fine specificity of those mAbs has been characterised at the epitopic level. Therefore, their binding to the allergen masks a particular known epitope and makes it unavailable for recognition by IgE antibodies.

Materials and methods

Allergic patients to peanut

Peanut-allergic children were recruited at the Department of Paediatric Allergy of the Hopital Necker-Enfants Malades (Paris, France). Two groups were identified. One comprised 23 patients with a confirmed peanut allergy based on positive oral provocation test and with high anti-whole peanut protein-specific IgE levels much above 0.3 IU/mL (Table 1). Sera from patients of this group were used to correlate the IgE values measured using different assays, e.g. the CAP^R system and in-house EAST tests. Four sera from this group, i.e. sera nos. 39, 44, 51 and 430, were also used for inhibition studies.

The other group was composed of 18 patients with indications of peanut allergy based on clinical observations, including a positive oral provocation test but with anti-whole peanut protein-specific IgE antibodies either not detectable or present at very low concentration (e.g. around or below 0.3 IU/mL) using conventional assays.

In addition, a control group of 44 patients with no allergy to peanut was included.

Preparation and purification of Ara h2 and Ara h6

Ara h2 and Ara h6 were purified from the WPPE as previously described [15]. Purified Ara h2 contained the two classical isoforms at 16.67 and 18.05 MW. Purified Ara h6 was composed of the native isoform, i.e. a 14.84 kDa protein containing five disulfide bridges and of a plant processed isoform, which is characterised by the loss of a dipeptide and the formation of two subunits linked by disulfide bridges as already described [15].

Anti-Ara h6 monoclonal antibodies

Anti-Ara h6 mAbs were raised in mice using a classical procedure already described for other antigens [16].

Table 1 IgE responses in 23 patients allergic to peanut

Serum no.	Anti-WPPE IgE (UI/mL) using CAP ^R system	Anti-WPPE IgE (UI/mL) using conventional in-house EAST	Anti-WPPE IgE (UI/mL) using reverse in-house EAST	Ratio anti-peanut protein-specific IgE/total IgE (%)
39	>100	>100	>100	ND
44	>100	>100	>100	ND
51	>100	>100	>100	ND
144	5.42	16.3	14.8	0.61
181	0.41	1.3	0.8	0.06
248	15.9	15.5	0.8	0.41
255	5.09	5	7.6	11.2
259	0.95	0.87	1.1	0.43
265	27.6	17.8	21	2.9
396	0.44	0.3	0.7	0.22
406	0.73	0.73	0.75	0.83
407	3.65	3.5	4.4	8.0
415	12.4	9.4	10	1.9
430	>100	>100	>100	ND
441	44.1	48.2	45	2.5
448	12.4	17.8	20.2	1.7
449	20.1	21.9	23	5.2
555	17.3	13.6	28	3.7
568	2.05	2.7	1.5	3.5
599	1.23	1.3	0.6	ND
635	4.72	4.04	14	ND
652	3.27	2.2	7.9	2.4
677	1.09	1.6	1.4	0.12

ND not determined, WPPE whole peanut protein extract

Native Ara h6 was used as immunogen. The monoclonal antibodies produced were screened on micro-titre plates coated with goat anti-mouse antibodies using Ara h6- and Ara h2-AChE conjugates as tracers. Positive hybridoma cells were cloned and expanded as ascitic fluids in nu/nu mice. Monoclonal antibodies were tested for their reactivity towards the two isoforms of Ara h6 and Ara h2. Finally, the specificity of mAbs was further characterised using peptide fragments of Ara h6 according to the procedure already described with another allergen [17].

Interactions of peanut proteins with IgE from allergic patients

Conventional EAST using immobilised allergens

IgE responses to peanut proteins were quantified using an EAST in which the whole peanut protein extract or purified peanut allergens were passively adsorbed on micro-titre plates according to Bernard et al. [4].

An EAST inhibition assay, based on a similar procedure, was used to study the inhibition of the IgE binding to immobilised allergens using increasing concentrations of the different purified allergens as competitors.

Reverse EAST using immobilised anti-IgE monoclonal antibodies and labelled allergens

Preparation of labelled allergens

Tracers were prepared by covalent linkage of the purified proteins to the tetrameric form of AChE according to Caruelle et al. [18].

Preparation of immunopurified IgE specific to whole peanut proteins

IgE antibodies specific to whole peanut proteins were immunopurified from peanut-allergic patient sera using whole peanut protein extract coupled to sepharose and quantified by the conventional immunoassay previously described [4].

Reverse EAST

Micro-titre plates were coated with the anti-human IgE monoclonal antibody LE27. Fifty microliters of different dilutions of individual serum from each patient were distributed and incubated overnight at 4 °C. After washing, 50 µL of AChE-labelled whole peanut protein extract or purified proteins (e.g. Ara h2 or Ara h6) were dispensed and incubated for 4 h at room temperature.

After washing, Ellman's reagent was used as the enzyme substrate, absorbance was measured, and results were expressed as mDO for response to Ara h2 and Ara h6, whereas they were quantified as IU/mL for response to whole peanut protein extract, using known quantities of purified anti-peanut IgE as internal standards.

Inhibitions of the reverse EAST were performed using the same procedure but in addition, 50 µL of inhibitors (i.e. increasing concentrations of purified Ara h2 or isoforms of Ara h6) were dispensed at the same time as the tracer. Results were expressed as B/B_0 , where B_0 and B represent the amount of labelled Ara h2 or Ara h6 bound to the immobilised IgE antibodies in the absence or presence of a known concentration of inhibitor, respectively.

Characterisation of IgE-binding epitopes on Ara h6 using anti-Ara h6 monoclonal antibodies

Different anti-Ara h6 monoclonal antibodies of characterised specificity were used to coat micro-titre plates using the same procedure as for peanut proteins. Plates were washed before use, and 100 µL of native Ara h6 isoform at a 10 µg/mL concentration were dispensed in each well. After a 4-h incubation at room temperature, plates were extensively washed, and various dilutions of individual sera from allergic patients were added. After 24-h incubation at 4 °C, specific IgE antibodies bound to the immobilised Ara h6 were measured using the anti-IgE monoclonal antibody BS17 labelled with AChE as a tracer as described before.

Using this format of EAST, the binding of specific IgE antibodies is hampered if the IgE specificity is the same as that of the characterised mAb used as capture antibody, e.g. if both recognise the same epitope on the Ara h6 molecule. On the opposite, if a significant binding is observed, this would mean that the IgE antibodies or most of those present in the polyclonal population of IgE antibodies of an allergic patient serum are not directed against the same epitope as the capture mAb used and therefore that the corresponding region of the molecule does not encompass a major IgE-binding epitope.

All the determinations were made on triplicate.

Results and discussion

Comparison of conventional and reverse EAST

In the reverse EAST, the capture of IgE antibodies is performed with the anti-human IgE monoclonal antibody LE27 coated on the solid phase. This monoclonal antibody is directed against the Dε2 domain of the IgE [19] and should not interact in the subsequent binding of specific IgEs to the allergen.

The enzyme AChE used as a label is characterised by a high specific activity. Furthermore, the enzymatic hydrolysis of the Ellmann reagent leads to a yellow-coloured compound in which absorbance at $\lambda=414$ nm is proportional to the time of reaction, which allows a good sensitivity of the assay.

With regards purified allergens, coupling AChE to Ara h2 and Ara h6 was made in well-defined conditions that allowed to estimate at 1 and 1.5 the number of AChE molecules linked per molecule of Ara h2 and Ara h6, respectively. However, variations may occur during the several steps of the preparation of the labelled proteins and result in variations of the enzymatic specific activity of the different tracers. Whole peanut protein extract contains numerous allergens, and the labelling of each one may vary depending on its structure. Such variations in the specific activity hamper an absolute comparison between the signal intensity (e.g. absorbance) obtained with the different allergen tracers.

In order to validate the different EAST assays, we compared the determinations of the specific IgE antibodies obtained using our home-made conventional EAST with those obtained using the commercial CAP^R system, on 23 patient sera with high anti-peanut protein IgE concentrations. The calculated correlation values were high, i.e. $r>0.80$, $p<0.001$ for concentrations of specific IgE to WPPE and $r>0.75$, $p<0.001$ for concentrations of specific IgE to purified allergens. In addition, the determinations of specific IgE antibodies to purified 2S albumins obtained using conventional and reverse EAST were also well correlated, i.e. $r>0.85$, $p<0.001$ (Table 1).

We have then used the reverse EAST assay to measure the IgE-binding capacity of whole peanut protein extract using sera from 18 patients with a clinically confirmed peanut allergy but with undetectable or very low concentrations (below 0.3 IU/mL) of specific IgE as measured by the conventional EAST. In a second time, the IgE-binding capacity of purified 2S peanut albumins was analysed.

As shown in Table 2 on four representative sera, the signals observed in the conventional EAST using immobilised WPPE were very close to the non-specific binding (background) whatever the dilution of the sera was. Conversely, the signals were much increased in the reverse

Table 2 Ratios between the values of the anti-WPPE specific IgE antibodies signals and the non-specific binding (background) as measured using conventional EAST assay (using WPPE immobilised on the micro-titre plates and AChE-labelled anti-human IgE mAbs) or

reverse EAST assay (using immobilised anti-human IgE mAbs and AChE-labelled WPPE) on three dilutions of four representative allergic patient sera with very low specific IgE values, close to the limit of detection

Serum no.	37		41		105		276	
EAST used	Conventional	Reverse	Conventional	Reverse	Conventional	Reverse	Conventional	Reverse
Serum dilution 1/5	2.8±0.2	42±1.5	2.7±0.2	11.5±1	3.2±0.2	17±0.6	1.6±0.1	10±0.8
Serum dilution 1/10	2.2±0	21±1.3	2.2±0.1	6.2±0.7	2.4±0.1	10.5±0.6	1.6±0.1	5±0.2
Serum dilution 1/20	1.8±0.1	8.5±1.3	1.7±0.	2.6±0.7	2±0.1	5.1±0.9	1.5±0	3.4±0.2

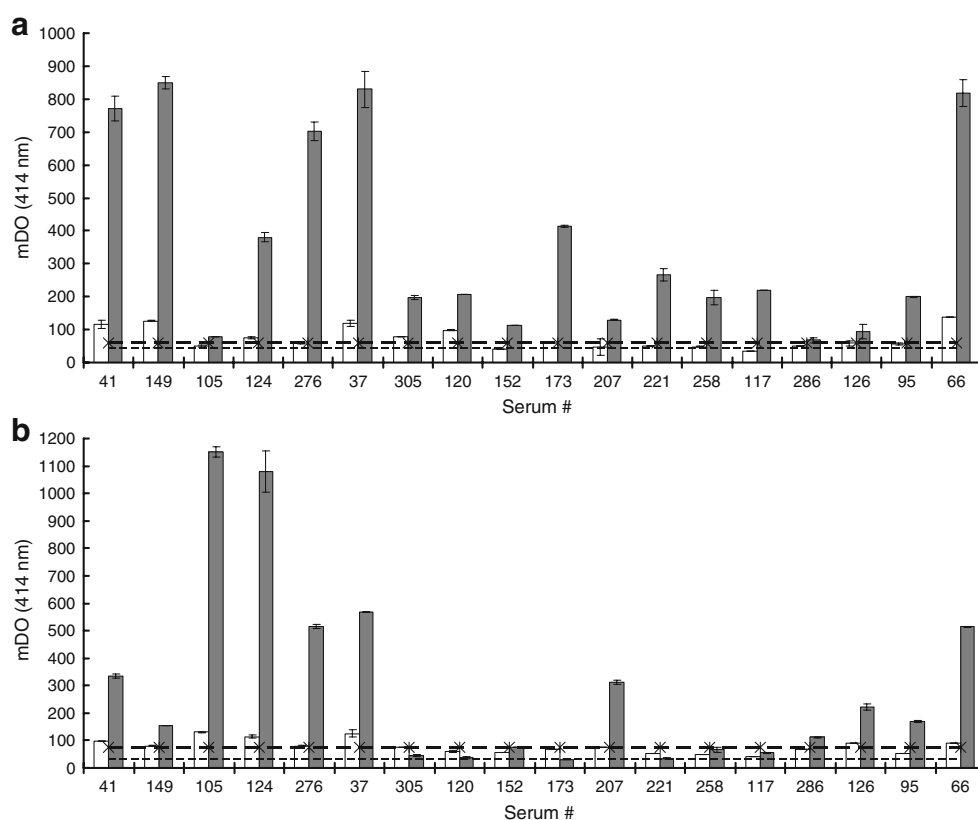
EAST and became significantly higher than those observed with the negative control even at a 1:20 dilution. The reverse EAST using a capture of IgE antibodies as a first step made the determination possible on diluted sera (up to 1:20) with a good linearity between the dilution and the signal and with a high signal/background ratio, which increases the sensitivity of the test.

The allergenicity, i.e. the IgE-binding capacity of purified Ara h2 and Ara h6, has then been analysed on individual sera from 18 patients with very low specific IgE titers using either conventional or reverse EAST (Fig. 1). A majority of them (i.e. 11 out of 18) presented no or very low IgE responses to immobilised Ara h2 or Ara h6. They

were close to the LoD, i.e. mean background calculated on the control group plus 3 standard deviation. In contrast, with the reverse EAST assay, labelled Ara h2 and Ara h6 were recognised by immobilised IgE antibodies of 18 and 17 allergic patient sera, respectively. The IgE binding observed were much higher than the LoD and highly significant with a 99% confidence level. The increased intensity of the signals made it possible (1) to use more diluted serum samples and (2) to develop an inhibition test (see below).

All of those patients who underwent a positive oral provocation test but had no or very weakly positive EAST tests were found significantly positive to Ara h2 or Ara h6

Fig. 1 IgE-binding capacity of Ara h2 (a) and Ara h6 (b) as measured by direct (□) or reverse (■) EAST assays using sera from 18 peanut-allergic patients with very low concentrations of anti-peanut protein-specific IgE. Non-specific binding (background) in the direct (— * —) and reverse (---) EAST assays



with the reverse EAST assay, which was in agreement with the clinical manifestations observed in those patients and could thus be used for the diagnostic of peanut allergy.

In both conventional and reverse EAST assays, none of the sera from control non-peanut-allergic patients gave false-positive result. On the opposite in the test population of peanut-allergic patients with low concentrations of specific anti-peanut IgE antibodies, the amounts of false-negative results were 11 of 18 (i.e. 61%) for both Ara h2 and Ara h6 using the conventional EAST assay vs 0 and one of 18 (i.e. 5.6%) for Ara h2 and Ara h6, respectively, using the reverse EAST assay.

In the reverse EAST assay, an extensive washing of the micro-titre plates is performed, after the step of capture of IgEs, before adding the labelled allergen. Therefore, even high levels of anti-peanut protein-specific IgG antibodies did not interact in the binding of allergen to immobilised IgE antibodies and interfere with the assay, neither did the presence of high concentration of non-specific IgE antibodies in some sera: the capacity of the solid phase to capture total IgE antibodies and the affinity of specific IgE to peanut allergens appeared sufficient to obtain significant signals (i.e. $>\text{non-specific background} + 3 \times \text{standard deviation}$) even when the ratio of anti-peanut-specific IgE/total IgE antibodies was much below 1% (Table 1).

It is noteworthy that although the binding of Ara h2 and Ara h6 to IgE antibodies generally occurs concomitantly in numerous peanut-allergic patients, the relative intensity and probably affinity of the binding of each allergen vary depending on the allergic patient. In order to further explore whether the high correlation observed between the IgE-binding capacity of Ara h 2 and Ara h 6 was due to polysensitisation and/or immuno-cross-reaction, we performed competitive inhibition tests with the two 2S albumins using the reverse EAST assay. Inhibition curves were plotted using four patient sera, and those obtained with serum from patient no. 51 are presented in Fig. 2a and b. They show that increasing concentrations of purified Ara h2 and also of the two isoforms of Ara h 6 were able to inhibit the binding of specific IgE to labelled Ara h2. The different curves were parallel but the inhibitions were higher when Ara h2 was used as competitor than when processed or native Ara h6 were used. Furthermore, the processed Ara h6 isoform appeared to be a better competitor than the native isoform (Fig. 2a). The IgE binding to labelled Ara h6 was totally inhibited by the two isoforms of Ara h6 and only partially inhibited by Ara h2 (Fig. 2b).

The corresponding IC_{50} values (e.g. concentrations of competitor that inhibit 50% of the IgE binding to the labelled allergen) varied depending upon the patient serum as shown in Table 3. In the case of reverse EAST inhibition assays using labelled Ara h2, IC_{50} values were 2- to 40-fold

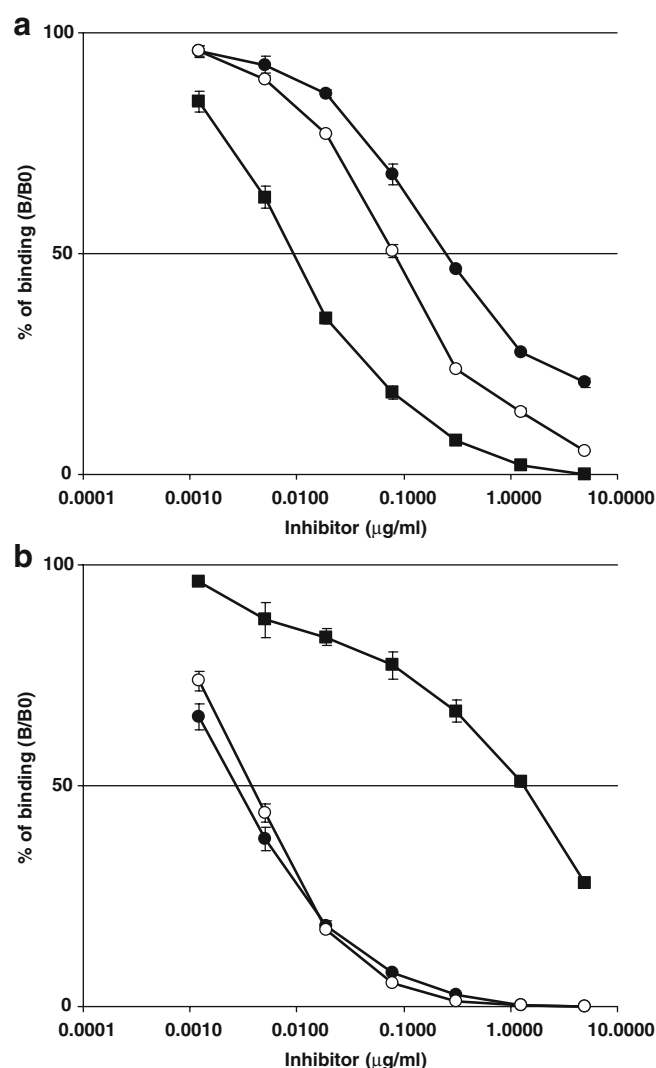


Fig. 2 Inhibition of the binding of Ara h2 (a) and of Ara h6 (b) labelled with AChE to the IgE antibodies of patient no. 51 by different competitors: filled squares Ara h2, filled circles native Ara h6, empty circles processed Ara h6

higher with the processed Ara h6 and 20- to 80-fold higher with native Ara h6 than with Ara h2 compared to the conventional assay, respectively. When inhibitions of the IgE binding to labelled Ara h6 were performed, IC_{50} of Ara h2 were up to ca. 200 times higher than those of both isoforms of Ara h6. Interestingly, in the conventional EAST, Ara h2 appeared to be a more potent competitor to inhibit the binding of IgE to immobilised Ara h6 comparing the reverse assay in which it inhibits the IgE binding to labelled Ara h6 in solution. A cross-reactivity is thus generally observed between peanut 2S albumins, the results obtained using the different formats of EAST illustrate the importance of the allergen presentation on its IgE-binding capacity and on the specificity and affinity of the binding.

Besides passive adsorption, different methods have been proposed to immobilise the allergen onto the solid phase in

Table 3 IC₅₀ (ng/mL), i.e. concentrations of competitors (Ara h 2, native Ara h 6 or processed Ara h 6) necessary to inhibit 50% of the binding of Ara h 2 or Ara h 6 to IgE antibodies from sera of four peanut-allergic patients as measured using conventional and reverse EAST inhibition assays

	Format of EAST inhibition used	Reverse Ara h2–AChE Anti-IgE-coated	Reverse Ara h6–AChE Anti-IgE-coated	Conventional Anti-IgE–AChE Ara h2-coated	Conventional Anti-IgE–AChE Ara h6-coated
Serum no. 430	Ara h2	20±0	400±130	15±4	500±200
	Native Ara h6	400±70	2±1	5±2	5±1
	Processed Ara h6	50±20	3±1.5	5±2	5±1
Serum no. 39	Ara h2	5±3	400±280	15±0	10±0
	Native Ara h6	350±100	10±1	100±70	80±40
	Processed Ara h6	200±40	10±1	80±14	50±20
Serum no. 44	Ara h2	10±4	300±140	10±1	5±0
	Native Ara h6	500±70	3±2	200±35	50±14
	Processed Ara h6	200±0	3±2	50±20	25±10
Serum no. 51	Ara h2	10±0	1,300±40	50±30	5±4
	Native Ara h6	300±140	4±1	500±70	80±50
	Processed Ara h6	100±70	5±1	50±8	20±10

the conventional format of EAST. The covalent immobilisation of proteins on photo-activated polystyrene plates has been described to enhance the sensitivity of the assay [20]. More recently, new techniques performed on various support using fixed allergen were generated to analyse the specific IgE response [21, 22].

Synthesis of peptides bond on membrane has been used for epitope mapping of the peanut allergens Ara h1, Ara h2, and Ara h3 [13]. However, this approach may not be appropriate for identification of conformational epitopes such as in Ara h6 in which the conservation of the conformation is crucial for the IgE-binding capacity. Indeed, we have observed that the IgG- and IgE-binding capacity of Ara h6 highly depends on its folding and that it

is decreased after reduction of the disulfide bridges [15, 23]. Therefore, we developed a method based on the presentation of the allergen that masks a given epitope for IgE recognition. It requires the immobilisation of the protein through interactions with anti-Ara h6 monoclonal antibodies of known epitopic specificity that are coated onto the solid phase.

As observed in Fig. 3, the binding capacity of Ara h6 to specific IgE from allergic patient sera is higher when Ara h6 is passively adsorbed than when it is presented by different monoclonal antibodies. When Ara h6 is presented either by mAb 633 or mAb 606, its binding capacity to IgE of a majority of sera was around 80 or 90% of that measured when it is passively adsorbed. Those two mAbs are known to be specific of the region of Ara h6 molecule, which includes fragment f(34–47). Therefore, we can conclude that this region does not encompass a major IgE-binding epitope for the population of patients studied because when it is masked for IgE recognition, the overall polyclonal IgE response is not significantly decreased.

By contrast, an important decrease of the IgE-binding capacity was observed in most sera when Ara h6 was bonded to mAb 201, which is an anti-Ara h6 monoclonal antibody that cross-reacts with Ara h2. The use of other anti-Ara h6 monoclonal antibodies cross-reacting with Ara h2 confirmed this result. This suggests that the homologous molecular domain(s) in peanut 2S albumins encompass major IgE epitope(s) and are strongly involved in peanut allergenicity.

In conclusion, reverse enzyme immunoassays and particularly enzyme allergeo-sorbent tests based on the capture of IgE antibodies as a first step of the assay appear to be an efficient tool to analyse the IgE-binding capacity of

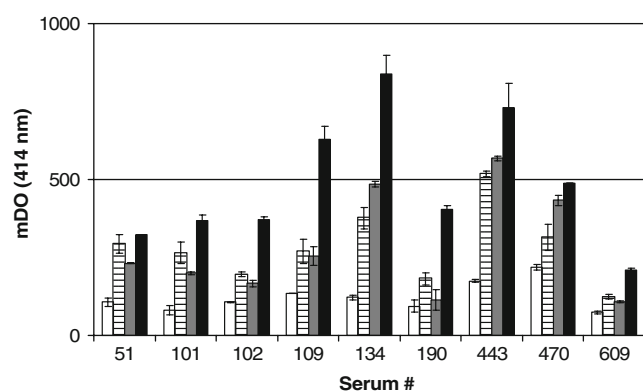


Fig. 3 IgE binding to coated Ara h6 using EAST assays in which Ara h6 is passively adsorbed on the solid phase (micro-titre plate) or immobilised after binding to different anti-Ara h6 monoclonal antibodies coated on the micro-titre plates. ■ Passive adsorption of Ara h6 (10 µg/mL). □ Presentation of Ara h6 by mAb 201. ▨ Presentation of Ara h6 by mAb 606. ▩ Presentation of Ara h6 by mAb 633

allergens and to characterise the structures responsible for this binding particularly in the case of peanut proteins. It is more sensitive than the conventional test using immobilised allergens and would avoid false-negative results when used as diagnostic tool of peanut allergy.

The reverse EAST inhibition assay also appears to be particularly efficient and accurate to analyse the specificity and/or affinity of allergen–IgE interactions, avoiding the interferences with other competitors such as specific IgG antibodies.

2S albumins such as Ara h2 and Ara h6 are most frequently involved in peanut allergy. With the reverse format of EAST, the use of purified Ara h2 and/or Ara h6 increased the specificity of the detection with a similar sensitivity as for the whole peanut protein extract and allows the standardisation of the test with a good reproducibility. In addition, the use of the recombinant well refolded Ara h6, which presents the same IgE immunoreactivity as the natural Ara h6 [23] can facilitate the standardisation and use of this assay for component-resolved diagnostics of peanut allergy.

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