



# NEW HORIZONS — ALLERGY —

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## The diagnostic value of IgE antibody measurements to peanut allergen components

### Summary

Peanut food allergy is a major public health problem because of its severity and prevalence, which is estimated to be 0.5% to 1.8% depending on the population group studied. Peanut is the most common food to cause fatal and near-fatal food allergy.

Useful diagnostic tests for food allergy are *in vitro* serum food-specific IgE assays, skin-specific IgE determination, basophil activation tests and oral food challenges.

Currently, the only way to assess a peanut sensitization is the use of native peanut extracts. Because of variability of the raw material linked to its origin and conditions of production and storage, investigators are confronted with a lack of standardization of the material used both for *in vitro* and *in vivo* testing. Production of recombinant allergens is a promising way to obtain biological material with consistent and standardized properties and will enable further characterization of the peanut-allergic patient.

Utilizing the recombinant allergens Ara h 1-3, rAra h 8, a Bet v 1-homologous panallergen, as well as nsLTP (rPru p 3), will be of value in the assessment of peanut allergy.

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### Introduction

Peanut food allergy is a major public health problem because of its severity and prevalence, which is estimated to be 0.5% to 1.8% depending on the population group studied.<sup>1,2,3</sup> Peanut is the most common food to cause fatal and near-fatal food allergy.<sup>4,5,6</sup> In addition, peanut allergy is characterized by its persistency.<sup>7</sup> As peanut allergy is relatively common, typically permanent, and often severe, it has attracted the most research attention.<sup>8</sup>

The threshold of clinical reactivity can be very low: Peeters *et al.* reported that the eliciting dose for subjective reactions varied from 0.1 mg up to 300 mg peanut, and from 10 to 3000mg for objective symptoms.<sup>9</sup> 18% of patients react at a dose less than 65 mg.<sup>10,11</sup>

### Peanut allergy diagnosis

The clinician may be faced with diagnosing peanut allergy in several circumstances, including a patient who is suspected of having had a reaction to peanut based on clinical history, a

child who has not ingested peanut but because of other food allergies or atopic disease was tested for peanut IgE, a previously peanut allergic child who may have outgrown the allergy, or to potentially avoid life-threatening peanut challenges.

Currently the useful diagnostic tests for food allergy are skin prick test (SPT), *in vitro* serum food-specific IgE assays, basophil activation tests and oral food challenges. These tests are based on natural peanut extracts that contain both allergenic and non-allergenic molecules. The extract composition depends on the origin of raw material and extraction, purification, and storage procedures. This leads to a great deal of variability and difficulty of standardization.<sup>12,13</sup> Production of recombinant peanut allergens leads to standardized reagents that are biochemically characterized and that can be produced on a large scale. Furthermore, recombinant peanut allergens may in the future enable the identification of specific peanut allergens which are clinically

relevant, or identify specific peanut allergens that are predictive of severity of the disease. Identification of IgE antibodies to specific peanut allergens may be predictive of other cross-reactive foods or even whether peanut allergy will persist or will be outgrown. Identification of sensitization to peanut allergen components might direct tailored specific immunotherapy in a next future.

*In vitro* methods (ImmunoCAP®, immunoblot methods, or ELISA) demonstrate the capacity of serum IgE (sIgE) binding of patients with allergy to allergens. Elevated levels of sIgE antibodies (abs) indicate and characterize patient biological sensitization status. The level of sIgE abs has been proposed to indicate the risk of food allergy (FA) in patients, in particularly with atopic dermatitis: a level higher than 15 kU<sub>A</sub>/l has a positive predictive value superior to 95%.<sup>14</sup> If sIgE ab levels are between 0.35 kU<sub>A</sub>/l and 15 kU<sub>A</sub>/l, however, it is not possible to predict accurately the risk of FA. An undetectable peanut-specific IgE level may correspond to a true FA in 27% of the cases, a level of sIgE <2 kU<sub>A</sub>/l to a 33% chance of a true FA, and a level <5 kU<sub>A</sub>/l to a 39% chance of FA.<sup>15</sup> The level of sIgE abs does not correlate with the severity of allergy and cannot be used as a predictive tool of severity.<sup>16</sup> The difficulty of interpretation of the level of IgE abs may be explained in part by the variability of the natural extract used for the biological tests, the presence of allergenic and non-allergenic molecules and carbohydrate, the techniques used, and the studied population.

However, merely detecting sensitization is not necessarily diagnostic of clinical allergy. This observation underscores the importance of the clinical history.<sup>8,17</sup>

Furthermore, these biological tests cannot currently be substituted for the oral challenge test that is the current standard for FA diagnosis. A better standardization of biological tests using recombinant allergens could allow the improvement of their diagnostic value.

## Peanut allergens

Peanut contains up to 32 different proteins, of which at least 18 have been identified as being capable of binding allergen-specific IgE.<sup>18,19</sup> Varieties of peanuts from different parts of the world contain similar proteins, and the IgE-binding properties have also been reported to be similar to a great extent.<sup>20</sup>

The International Union of Immunological Societies Nomenclature Subcommittee recognizes 8 allergenic proteins in peanut, from Ara h 1 to Ara h 8, although Ara h 3 and Ara h 4 are nearly identical isoforms and Ara h 6 is highly homologous to Ara h 2.<sup>21,22</sup> A list of peanut allergens characterized is listed in *Table 1*.

**Table 1.** Peanut allergens characterized to date.

<b>Ara h 1</b>	a 7S vicilin-like globulin <sup>23</sup>
<b>Ara h 2</b>	a 2S albumin, a conglutin seed storage protein, a trypsin inhibitor <sup>24</sup>
<b>Ara h 3</b>	an 11S globulin, a glycinin, a trypsin inhibitor <sup>25</sup>
<b>Ara h 4</b>	a glycinin <sup>26</sup>
<b>Ara h 5</b>	a profilin <sup>27</sup>
<b>Ara h 6</b>	a conglutin, 2S albumin <sup>27</sup>
<b>Ara h 7</b>	a conglutin <sup>27</sup>
<b>Ara h 8</b>	a Bet v 1-homologous allergen <sup>28</sup>
<b>Ara h Agglutinin<sup>29</sup></b>	
<b>Ara h LTP</b>	a lipid transfer protein <sup>30</sup>
<b>Ara h Oleosin<sup>31</sup></b>	

The 3 major allergens, Ara h 1-3, are comprised of a vicilin, conglutin, and glycinin seed storage proteins, respectively.<sup>32</sup> Two of the 8 identified peanut allergens are not storage proteins but proteins associated with pollen-associated food allergy; Ara h 5 is a profilin and Ara h 8 is a Bet v 1-like protein.<sup>26,28</sup>

Ara h 1, Ara h 2, and Ara h 3 are the 3 main allergens.<sup>33</sup> Ara h 1 comprises 12% to 16% of the total protein in peanut. In population studies, sensitization to Ara h 1 was found in 95% of peanut-allergic patients from North America,<sup>34,35,36,37</sup> but in fewer peanut-allergic patients of 3 European populations varying from 35% to 70%.<sup>27,38,39</sup> These differences have not been found for Ara h 2, even though peanuts from different varieties and from different parts of the world contain similar proteins and the IgE binding properties are similar.<sup>20</sup> Unidentified peanut proteins with molecular weights somewhat lower than 15 kDa may be important allergens as well.<sup>40</sup> Ara h 3 is recognised by serum IgE abs from 45% - 50% of patients with peanut sensitivity.<sup>41</sup> Ara h 5 shows up to 80% amino acid sequence identity with the panallergen profilin, but present only in low amounts in peanut extracts. 13% to 16% of peanut-allergic individuals are sensitized to peanut profilin.<sup>42</sup>

Significantly, studies demonstrate that sensitization to peanut occurs with a high degree of heterogeneity to a number of peanut allergens. Mono-sensitization to a single peanut allergen is relatively rare.<sup>33</sup> Therefore, although sensitisation to Ara h 1 and Ara h 2 occurs in the great majority of peanut-allergic individuals, the wide range of allergens present in whole peanut protein extract appears to be most appropriate to consider when testing for peanut allergy.<sup>37</sup>

This is illustrated by a number of studies. In a European study of peanut-allergic individuals, sensitization to Ara h 2 was found in 85%, to Ara h 4 in 53%, to Ara h 5 in 13%, to Ara h 6 in 38%, and to Ara h 7 in 43% of the selected sera.<sup>27</sup> Similarly, in a British study evaluating sera of 40 peanut-allergic individuals, of 18 allergens identified, 8 were bound by >50% of patients. The study concluded that promiscuity of IgE binding appears more important than the recognition of individual proteins.<sup>43</sup> Shreffler *et al*<sup>33</sup> demonstrated using *in vitro* studies that 97% of subjects with peanut allergy recognized at least 1 of these allergens, whereas 77%, 75%, and 77% recognized rAra h 1, rAra h 2, and rAra h 3, respectively.

Furthermore, some peanut-allergic subjects fail to bind to either Ara h 1 or 2 suggesting that whole peanut, rather than Ara h 1 or 2, or the use of individual peanut allergens would in general be more appropriate for measuring specific-IgE responses, but also illustrates that the relative contribution of all peanut allergens needs to be investigated and suggests the value of recombinant allergens.<sup>37</sup>

## Recombinant peanut allergens

Recombinant peanut allergens available are listed in **Table 2**.

**Table 2.**

rAra h 1. <sup>44</sup>
rAra h 2. <sup>45</sup>
rAra h 3. <sup>46</sup>
rAra h 8. <sup>47</sup>

Before peanut recombinant allergens can be used as a diagnostic tool, their immunologic reactivity and ability to induce skin reactions need to be established and compared with natural allergens.<sup>48</sup> Recently Astier *et al*<sup>49</sup> reported the results of a validation step on the basis of *in vitro* and *in vivo* methods of the 3 major recombinant peanut allergens, rAra h 1, rAra h 2 and rAra h 3, concluding that SPT using these three to be a safe and effective diagnostic tool. Furthermore, they reported that co-sensitization to rAra h 2 and rAra h 1 and/or rAra h 3 to be more predictive of more severe reactions.

## Predictive value of recombinant peanut allergens

Until the study of Astier *et al*,<sup>49</sup> the diagnostic value of skin tests to recombinant peanut allergens had not been established. The study evaluated the recognition profile of peanut recombinant allergens (rAra h 1, rAra h 2, and rAra h 3) *in vivo* (SPT) and *in vitro* (sIgE abs) and compared native peanut and commercial extract in 30 patients allergic to peanuts.

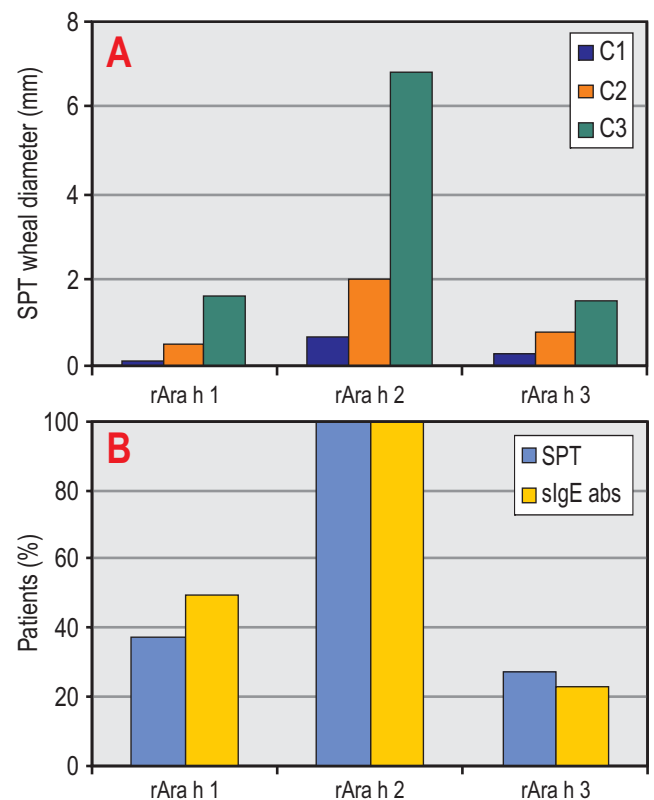
Thirty patients with peanut allergy (12 female and 18 male subjects), 15 nonatopic subjects, and 15 patients allergic to birch pollen without food allergy were enrolled. The mean age was  $9.2 \pm 0.8$  years (range, 3-20 years). The clinical severity of the allergy reaction was evaluated on a slightly modified scale of between 0 and 5 to that reported by Ewan and Clark.<sup>50</sup> The grading system used is shown in **Table 3**.

**Table 3.** Grading system for peanut-allergy clinical severity.

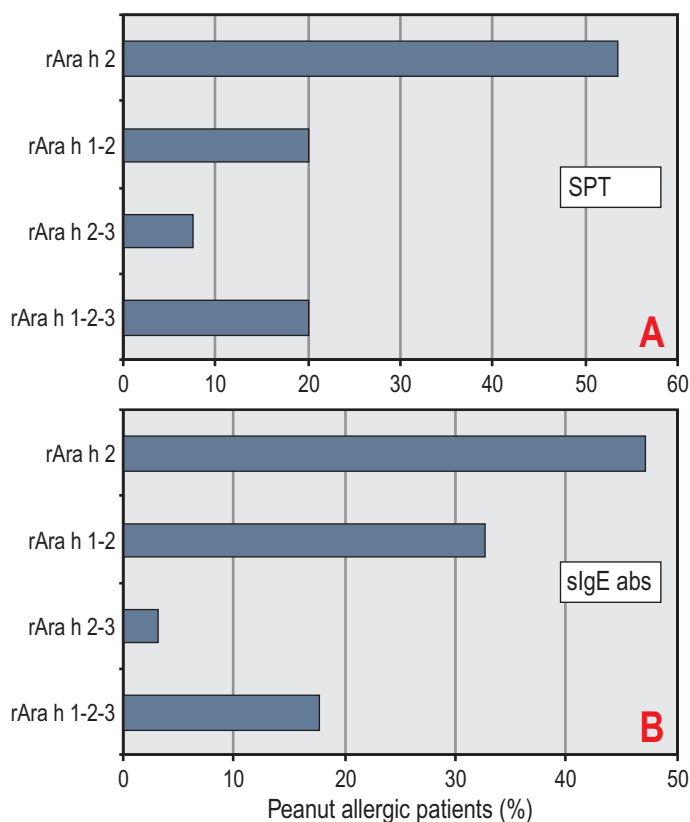
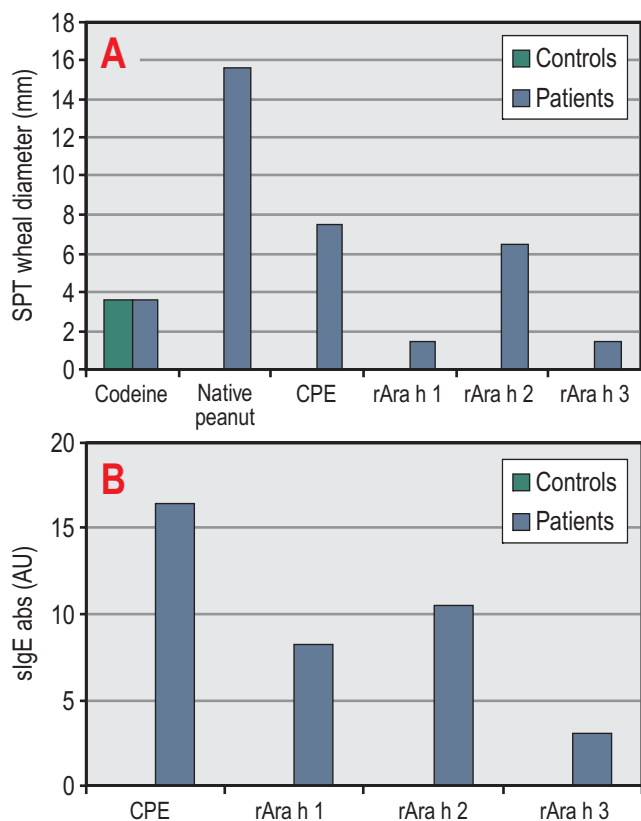
Grading	Symptoms
0	No symptoms.
1	Abdominal pain that resolved without requiring medical treatment, rhinoconjunctivitis, urticaria fewer than 10 papules, rash (eczema onset).
2	One organ involved, abdominal pain requiring treatment, generalized urticaria, non-laryngeal angioedema, mild asthma (cough or fall of peak expiratory flow < 20%).
3	Two organs involved.
4	Three organs involved or asthma requiring treatment or laryngeal edema or hypotension.
5	Cardiac and respiratory symptoms requiring hospitalization in intensive care.

The clinical features occurring after accidental ingestion were scored for 3 patients at grade 1, six others at grade 2, three at grade 3, and seven at grade 4. The double-blind placebo-controlled food-challenge (DBPCFC) induced symptoms of grade 1 in six patients, grade 2 in three, grade 3 in five, and grade 4 in three. Twenty patients presented cross-sensitization to other nuts, 10 patients to others legumes. Seven patients were monosensitized to peanuts.

No subject from the control group reacted with any recombinant allergen. One control subject with sensitization to birch pollen reacted by SPT to native peanut, with negative SPT result to commercial peanut extract (CPE) and recombinant allergens. On the other hand, rAra h 1, 2, and 3 were all able to induce cutaneous reactions characteristic of mast cell degranulation in patients allergic to peanut. Intensity of this reaction as determined by the wheal diameter increased concomitantly with the concentration of the preparation (**Figure 1, A**). At the highest concentration tested, all patients with peanut allergy reacted by SPT to rAra h 2, 40% to rAra h 1, and 27% to rAra h 3 (**Figure 1, B**). Native peanut gave a positive SPT result with papules significantly larger than those observed with CPE ( $15.4 \pm 1.2$  vs  $7.6 \pm 0.7$  mm;  $p < .001$ ). The size of the wheal resulting from the SPT with rAra h 2 was comparable to that of the size of the wheal resulting from the SPT with CPE (**Figure 2, A**).



**Figure 1.** Peanut recombinant allergen reactivity. **A:** SPT wheal diameters (mm). **C1**, 1 µg/ml; **C2**, 10 µg/ml; **C3**, 60 µg/ml for rAra h 1 and **C3**, 100 µg/ml for rAra h 2-3. n = 30. **B:** Patients (%) with positive SPT and sIgE abs to recombinant allergens. n = 30.



**Figure 2.** Reactivity to peanut extracts and peanut recombinant allergens.  
**A:** Wheal diameters (mm) obtained by SPT. rAra h 1, 60 µg/ml; rAra h 2 and 3, 100 µg/ml.  
**B:** Levels of sIgE abs determined by ELISA (n = 30 subjects/group).

Sixteen patients reacted only to rAra h 2 and 14 to more than 1 recombinant allergen. Out of 8 theoretical possible combinations, 4 were observed (Figure 3, A). The profile of sensitization did not differ between patients monosensitized to peanut and those polysensitized to legumes or other nuts.

There was no correlation between the wheal size of the SPT of rAra h 2 and the clinical score of severity (data not shown). However, patients who were monosensitized to rAra h 2 had a significantly lower severity score than polysensitized patients ( $p < .02$ ; Figure 3, C).

The profile of recognition of recombinant allergens by sIgE abs exhibited a similar pattern to that of the SPT. All patients had sIgE abs against rAra h 2, 50% against rAra h 1, 20% against rAra h 3 (Figure 1, B). The mean levels of sIgE recognizing CPE, rAra h 2, and rAra h 1 were comparable, whereas they were lower for rAra h 3 (Figure 2, B). sIgE binding to recombinant proteins followed a distribution pattern similar to that resulting from SPT (Figure 3, B). There was no correlation between the level of sIgE abs and severity of allergy. However, patients monosensitized to rAra h 2 had a less severe disease than patients who were polysensitized (Figure 3, D). The levels of sIgE abs were significantly higher in polysensitized than in monosensitized patients for both CPE ( $29.2 \pm 8.3$  vs  $5.2 \pm 3.9$  AU;  $p < .02$ , respectively) and rAra h 2 ( $17.3 \pm 5.1$  vs  $4.4 \pm 3.4$  AU;  $p < .04$ , respectively).

**Figure 3.** Spectrotypes of recombinant allergen reactivity and clinical score of patients monosensitized to rAra h 2 and polysensitized.  
 Spectrotypes as defined by SPT (A) and by sIgE abs (B). Peanut allergy severity in rAra h 2 monosensitized and polysensitized patients as defined by SPT (n = 16 rAra h 2 monosensitized and n = 14 polysensitized patients; C) and sIgE abs (n = 14 rAra h 2 monosensitized and n = 16 polysensitized patients; D).

## DISCUSSION

Currently, the only way to assess a peanut sensitization is the use of native peanut or commercial extracts derived from native peanut. Because of variability of the raw material linked to its origin and conditions of production and storage, investigators are confronted with a lack of standardization of the material used both in skin and biological testing.<sup>12, 13</sup> The technology of recombinant allergens appears to be a promising way to produce biological material with consistent and standardized properties that will enable further characterization of the peanut-allergic patient.

All patients with peanut allergy included in the Astier study<sup>49</sup> reacted to rAra h 2, and no false positives were observed. Thus, rAra h 2 appeared to be “the most interesting candidate” for use in the diagnosis of peanut allergy. The authors pointed out that the high sensitivity and specificity of SPT to rAra h 2 needed to be confirmed in a larger population. Skin reactivity to rAra h 2 at the concentration of 100 mg/ml was comparable to that to CPE. The allergenicity of Ara h 2 has been in part attributed to the high avidity of IgE abs for this protein.<sup>51</sup> The higher responsiveness of SPT to native peanut compared with commercial extracts has been previously observed,<sup>52</sup> which could be explained either by the cross-reactivity with carbohydrate determinants and some pollen or vegetable allergens.<sup>49</sup>

An important problem encountered with SPT to peanut is that positive SPT results to peanut are observed in pollen-sensitized patients.<sup>53</sup> This was highlighted in a recent study by Mortz *et al.*<sup>54</sup> The rate of sensitization was 5.8% for sIgE abs (ImmunoCAP<sup>®</sup>) and 3.4% for SPT whereas the diagnosis of true allergy was estimated at 0.5%. Ninety-six percent of the study group was sensitized to grass pollen. The high rate of sensitization to peanut detected by sIgE abs and SPT was attributed to the cross-reactivity between peanut and pollen allergens, which can be as a result of carbohydrate determinants or homology of allergenic proteins. This could have been the explanation for the positive SPT to native peanut in 1 control subject sensitized to birch pollen. In fact, cross-reactivity with birch pollen allergens (Bet v 1 and Bet v 2) have been demonstrated with Ara h 5 and Ara h 8.<sup>28</sup>

In this instance, recombinant allergens may be particularly of diagnostic value. Recombinant allergens produced in *E. coli* lack carbohydrate determinants thereby eliminating the risk of false-positive results because of these determinants.<sup>55</sup> Furthermore, the absence of carbohydrate determinants could affect refolding and thus the tertiary structure of recombinant proteins which would modify the recognition of recombinant allergens by sIgE compared with the wild-type allergen. This may be the explanation why there is no cross-sensitization with birch pollen allergen. The skin reactivity and recognition of recombinant allergens by sIgE abs is more reflective of epitope peptides, and it is not surprising that the findings of the Astier study<sup>49</sup> agree with those of Beyer *et al.*<sup>56</sup> and Shreffler *et al.*<sup>53</sup> concerning clinical reactivity and severity.

One of the main problems for physicians is having diagnostic tools sufficiently effective to evaluate the severity of the disease without the use of oral food challenge tests that require a specialized clinical platform and that are time-consuming. Sampson<sup>14</sup> has defined levels of sIgE abs for a number of foods with a positive predictive value of 95%, where a food challenge test can thereby be avoided. However, how to determine whether a patient with a peanut sIgE level less than 15 kU<sub>A</sub>/l has or does not have a true allergy to peanut is

an issue. Similarly, the threshold of SPT diameter with a predictive positive value of 95% has been defined as >8 mm for commercial extract<sup>57</sup> but no relationship has been demonstrated between the size of SPT or sIgE abs and the severity of the disease. A French study in children less than 16 years has showed that a size of SPT > 16 mm and a level of sIgE abs to peanut > 57 kU<sub>A</sub>/l is required to reach a PPV of 100%.<sup>58</sup> Polysensitization could be associated with the severity of symptoms had previously been proposed by Beyer *et al.*<sup>56</sup> and required exploration.

Using synthetic peptides, the authors demonstrated that the cumulative IgE binding to the peanut peptides was significantly higher in patients with peanut allergy than in tolerant patients. The recent study of Lewis *et al.*<sup>59</sup> showed that the total number of bands recognized by sIgE abs per patient is correlated with challenge score. Data from the Astier study<sup>49</sup> fully supported this conclusion: sensitization established either by SPT or sIgE abs to rAra h 1 and/or rAra h 3 in addition to rAra h 2 was associated with more severe symptoms. This offers the possibility of developing reagents predictive of disease severity that can be used by the practitioner. By bioinformatic annotation of sequences closely related to currently identified immunodominant allergenic epitopes,<sup>46, 60, 61</sup> epitopes were located predominantly on Ara h 2, whereas Ara h 1 and Ara h 3 contained fewer of these, which could explain why all subjects with clinically proven peanut allergy indeed reacted to rAra h 2. Additional reactivity to others, rAra h 1 and rAra h 3, was associated with high clinical scores, suggesting that disease severity is related to epitope spreading. The data was consistent with the higher epitope diversity found by Shreffler *et al.*<sup>53</sup> in patients with a history of more severe allergic reactions.

Peeters *et al.*<sup>6</sup> used purified peanut proteins (Ara h 1-3, Ara h 6) and found a correlation of clinical severity with recognition of Ara h 2 and 6 at low concentrations, and Ara h 1 and 3 at higher concentrations, indicating apparent increased potency of Ara h 2. The majority of patients with a positive SPT were sensitized to Ara h 2 (25/30, 83%) and Ara h 6 (26/30, 87%). Sixteen patients (53%) were sensitized to Ara h 1 and 15 patients (50%) to Ara h 3. All patients with a positive SPT to Ara h 1 and/or Ara h 3 were also sensitized to Ara h 2 and/or Ara h 6.<sup>9</sup> This was also noted by Palmer *et al.*<sup>62</sup> In the Astier study,<sup>49</sup> recombinant Ara h 1-3 was used and binding was found to be dominant to recombinant Ara h 2, but severity correlated with polysensitization. Indeed, a positive correlation of reaction severity with increased diversity of binding, whether to the allergens<sup>9, 43</sup> or epitopes,<sup>33</sup> is a common theme that may translate to future diagnostic tests that can predict severity, likelihood of current allergy, and resolution.

One of the main public health problems of FA to peanut is its persistency: the spontaneous recovery rate is estimated between 18% and 21.5%,<sup>7, 63</sup> and the recurrence rate after recovery at 7.9%.<sup>64</sup> Other than avoidance diet and emergency kits containing self-injectable epinephrine, the severity of symptoms, the deterioration of the quality of life, the risk of recurrence, and the weak percentage of spontaneous recovery have prompted different research teams to develop other therapeutic strategies.<sup>64</sup> Previous trials of immunotherapy using peanut extract demonstrated increased tolerance of patients with peanut allergy to oral ingestion of peanuts, but the high rate of repeated severe systemic reactions in most patients, even during maintenance injections, led to the cessation of this kind of treatment.<sup>65</sup>

## Conclusion

The Astier study<sup>49</sup> demonstrated that the profile of recognition of recombinant allergens by sIgE abs exhibited a similar pattern to that of the SPT. sIgE binding to recombinant proteins followed a distribution pattern similar to that resulting from SPT. Utilizing the recombinant allergens Ara h 1-3, and including rAra h 8, a Bet v 1-homologous panallergen<sup>28, 65</sup> may be of value in the assessment of peanut allergy.

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**NEW HORIZONS**

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