

Component-resolved diagnostics in food allergy

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Purpose of review

The purpose of this paper is to review and discuss recent studies on component-resolved diagnostics in food allergy, involving panels of pure allergen molecules or arrays of peptides derived from allergen sequences, and to summarize the reporting of new food allergens during the past 2 years.

Recent findings

Several component-resolved diagnostic studies in food allergy suggest that the use of panels of allergen molecules may allow refined clinical information to be obtained on the likelihood or severity of an allergic food reaction and regarding diagnostic specificity. Further, in some studies the use of pure allergen molecules has led to a clearly higher sensitivity of the immunoglobulin E immunoassay compared with conventional allergen extracts.

Summary

While common diagnostic methods in allergy assess the presence or absence of allergen-specific sensitization, to date, no in-vitro or in-vivo test exists which exhibits full correlation with clinical food allergy. A multitude of recently reported findings and observations indicate that molecular analysis of allergen sensitization pattern may serve to enhance the clinical utility of immunoglobulin E antibody-based allergy diagnostics. Pure natural and recombinant allergen molecules as well as panels of synthetic peptides have been used for this purpose.

Keywords

allergen panel, component resolved diagnostics, food allergen, IgE epitope, peptide microarray

Introduction

Food allergy is an important health problem within the general population. Recent epidemiological studies and data based on objective diagnostic methods indicate that 2–6% of the general population suffer from food allergy [1–5], making it one of the most common chronic disorders in modern society. The finding that up to 25% of the population claims a subjective perception of food allergy [3] indicates the magnitude of the problem and the need for appropriate diagnostic methods.

An accurate diagnosis in patients suffering from food-related symptoms is extremely important in order to clarify the nature and cause of the reactions and to enable effective measures of disease management. Dietary restrictions need to be relevant and unnecessary diets must be avoided in the interest of the patients' health and well-being. Thus, a reliable link needs to be established between the adverse reaction to food as reported by the patient and the immunological basis of this reaction.

Food allergic reactions range in severity from very mild and local symptoms, as in the oral allergy syndrome or contact urticaria, through associated systemic symptoms involving distal organs, to fatal outcome. The severity of a reaction may depend on a variety of factors, including amount of ingested allergen, type of allergen, the digestion and uptake of allergen in the gastrointestinal system, as well as cofactors such as the health status of the individual, concomitant viral infections, physical exercise, intake of alcohol, cold beverages or drugs such as β -blocking agents or nonsteroidal antiinflammatory drugs.

Huge strides have been made in identifying, characterizing, and cloning of allergens during the past decade [6] and this has resulted in increased knowledge of allergenic components in a large variety of plant-derived and animal foods. From early studies on food allergens it has become evident that, depending on the route of sensitization, food allergy may be either linked to inhalant allergens, as in the case of Bet v 1-related proteins, or may originate as a genuine food sensitization, unrelated to respiratory sensitization. Examples of 'genuine food allergens' include the major peanut allergens and other nut storage proteins.

Food allergens have been studied for their ability to resist gastric digestion and several recent reports suggest that allergenic determinants resistant to the gastric environment may be frequent causes of severe allergic reactions

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Abbreviations

CRD	component-resolved diagnostics
DBPCFC	double-blind placebo-controlled food challenge
Ig	immunoglobulin
LTP	nonspecific lipid transfer protein
SPT	skin prick testing

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to foods [7–9]. Thus, allergy to a particular food may give rise to differentially severe symptoms depending on which precise allergen component(s) the individual is sensitized to. For example, the predominant sensitization to nonspecific lipid transfer proteins (LTPs) in *Rosaceae* fruit-allergic individuals in the Mediterranean area is often accompanied by a history of systemic food reactions. This is rarely the case, however, in northern European populations where sensitization to *Rosaceae* fruits is characteristically directed to Bet v 1-related food allergens [10–12].

A different aspect of food allergy is its development over time, particularly in childhood. Different food allergies are known to be subject to either outgrowth or persistence in different proportions of individuals. While no clinical method has so far been described whereby outgrowth or persistence can be predicted in a given individual, Sampson and coworkers provided evidence in several reports [13–15,16^{••},17^{••},18] that the pattern of immunoglobulin (Ig)E antibody reactivity to synthetic peptides derived from milk and peanut allergens is associated with the level of persistence of these allergies throughout childhood and adolescence.

Yet another relevant aspect related to the component-specific sensitization pattern is the likelihood of an observable sensitization to cause clinical reactivity. While certain allergens are known to be strongly predictive of manifest allergic disease, others, typically cross-reactive determinants such as profilins or particular glycan structures, are considered only weakly associated to clinical reactivity [19,20].

Thus, a multitude of recently reported findings and observations indicate that molecular analysis of the allergen sensitization pattern may serve to enhance the predictive and prognostic power of IgE antibody-based allergy diagnostics. Although suggestions have been made on how to ascertain sensitization to specific allergen molecules from clinical parameters of individual patients [21[•]], this appears to be very difficult or even impossible. Accordingly, the increasing availability of allergen panels derived from various sources enables a detailed analysis of sensitization profile in individual patients. This concept has been defined as ‘component-resolved diagnostics’ (CRD) [22]. The purpose of CRD is to establish significant associations between specific subpopulations of specific IgE, measured by the use of individual allergen components or parts of these components, and clinically relevant aspects of the allergic disease. Examples of such allergen components useful for CRD studies include Mal d 1, 2, 3 and 4 from apple [23], Bos d 4, 5, 6 and 8 from cow’s milk [24[•]], Gal d 1, 2, 3 and 4 from hen’s egg [25[•]] or even panels of synthetic peptides, representing discrete epitopes derived from selected

allergenic molecules [14,16^{••}]. CRD can also be based on panels of homologous allergens sharing similar structures and a high degree of sequence identity, detecting different levels of co-recognition by specific IgE antibodies (e.g. Bet v 1 homologues from hazelnut, apple and celery or vicilins from different legume seeds).

An intrinsic advantage of CRD, through its use of purified natural or recombinant allergen molecules rather than crude natural extracts [26], is a superior diagnostic sensitivity which has been demonstrated in several cases. The deployment of such highly potent reagents in routine diagnostic tests would have an immediate positive effect on their diagnostic efficacy in clinical practice.

The present review is aimed at reviewing recent developments in the field of CRD in food allergy and critically discussing the potential, future perspectives and limitations of this approach.

Current diagnostic procedures

Common diagnostic methods used in allergy investigations assess the presence or absence of allergen-specific sensitization. Sensitization is a prerequisite for IgE-mediated allergy but is not always accompanied by allergy symptoms. Thus, whereas the absence of sensitization can be used to exclude allergy as a cause of observed symptoms, its presence is not sufficient for a positive diagnosis of allergy.

Skin prick testing (SPT) and in-vitro determination of food-specific IgE are still the primary tools for the diagnosis of food allergy in daily practice [27]. While SPT is an inexpensive and rapid method, the outcome of an SPT is influenced by a variety of factors that are difficult to standardize, such as use of commercially available extracts versus native foods, source of allergens, condition of the skin where the test is carried out, prick technique and concurrent intake of medication. Furthermore, many commercial food extracts that are used in the diagnosis of food hypersensitivity still lack appropriate biological standardization or a precise adjustment of the content of individual allergens. Natural extract preparations for in-vivo and in-vitro diagnosis contain a mixture of allergenic and nonallergenic components that are usually obtained from source materials by relatively simple buffer extraction procedures. Despite the fact that significant efforts have been made towards improving biological standardization of allergen extracts, variable composition is an inherent and unavoidable problem [28]. Some allergen content problems are specifically associated with food extracts, resulting in more severe quality issues in comparison to extracts of many aeroallergen sources such as pollens, mites or animal epithelia. For example, lability of some components and endogenous enzyme activities leading to degradation or inactivating

modification processes, as well as low abundance of some major food allergens, may result in suboptimal diagnostic performance of allergen extracts from foods.

Quantitative measurement of specific IgE to food allergen extracts has been reported to be useful in allergy diagnosis [29]. 'Decision points' of specific IgE levels have been correlated with outcome in food challenge tests to predict clinical relevant allergy [30]. Such decision point values have been defined for some foods (e.g. egg and peanut) [31] but not for others (e.g. wheat and soy) [29] but unfortunately inconsistent results were obtained when allergy to the same food was studied in different centers [32], possibly as a result of different age distributions of the patient cohorts studied. Therefore, food challenges, in particular the double-blind placebo-controlled food challenge (DBPCFC), represent the most reliable way to establish or rule out hypersensitivity to a food. New diagnostic approaches in food allergy must therefore be validated in patients with food allergy confirmed by a positive DBPCFC. While no procedural standardization of food challenges has been agreed upon, guidelines have recently been elaborated and published in a European position paper [33].

To date, no in-vitro or in-vivo test exists which shows full correlation with clinical food allergy and there is an urgent need for new and fundamentally improved diagnostic approaches available to a wider range of patients. It cannot be expected that the use of novel allergen reagents – such as purified or recombinant allergen components – alone will resolve the problem of sensitization not accompanied by clinical allergy, but improved clinical sensitivity is a realistic goal.

Component-resolved diagnostic studies in food allergy using pure allergen molecules

Several studies based on the CRD concept using pure allergen molecules have recently been published. The IgE antibody response in carrot allergy has been examined in detail by Ballmer-Weber and co-workers [34^{••}]. Forty patients carefully selected according to clinical history, specific IgE and a positive DBPCFC were enrolled. Two isoforms of Dau c 1 (Dau c 1.0104 and Dau c 1.0201), the profilin Dau c 4 and a reagent for cross-reactive carbohydrate determinants were used to assess sensitization, as were the birch pollen allergens Bet v 1 and Bet v 2 for comparative purposes. The data confirmed that Dau c 1 is a major allergen in carrot allergy. While sensitization to isoform Dau c 1.0104 was more prevalent among the individuals studied, isoform Dau c 1.0201 showed the best correlation to the clinical situation. In addition, the study provided evidence that a LTP previously shown to be produced in somatic embryo cultures of carrot [35,36] and thought to be relevant in carrot allergy [7,37,38] is not present at detectable levels in the edible parts of carrots.

The absence of this protein may explain why carrot allergy is very rare in Mediterranean countries where LTP sensitization is common.

In a different study on food allergy to apple, carrot and celery, Erdmann and collaborators [39] focused on a panel of Bet v 1 homologues from these foods, namely Mal d 1, Dau c 1.01 and Api g 1.01. In addition, Bet v 1 and Bet v 2 were included for comparative purposes. A functional basophil activation test (BAT) with pure allergen molecules was compared with ImmunoCAP measurements with whole allergen extracts, revealing clinical sensitivities and specificities of 60–75% and 64–86%, respectively, for the IgE assay and 65–75% and 68–100%, respectively, for the BAT assay. The patients, however, were not selected on the basis of challenge tests and in contrast to [34^{••}], no birch pollen allergic control group without food allergy was included for evaluation of diagnostic performance. The authors concluded that, although a better characterization of the study participants would have been relevant, the use of CRD and inclusion of biological assays such as BAT might help to identify the clinically most relevant allergens for in-vitro tests. Bet v 1 and Bet v 2 have also been included in a CRD study dealing with grass pollen allergy [40]. Recording of detailed case histories and determination of IgE reactivity to various pollen extracts as well as recombinant birch pollen allergens helped to define different subsets of grass pollen allergic individuals, with or without food allergy and with or without co-sensitization to birch pollen allergens.

Gly m 4 and Ara h 8 were recently identified as Bet v 1-related molecules in soybean and peanut, respectively, and produced in recombinant form [41,42[•]]. In addition to extending the panel of soybean and peanut allergens, Gly m 4 in particular facilitated a relevant improvement of diagnostic sensitivity when used as an ImmunoCAP reagent. While only 10 out of 22 (45%) individuals with pollen-related allergy to soybean tested positive with the soybean extract-based test, all but one (96%) showed IgE binding to rGly m 4 coupled to streptavidin-coated ImmunoCAP tests [42[•]].

Garcia-Ara and co-workers [43^{••}] reported that purified Bos d 4 (α -lactalbumin), Bos d 5 (β -lactoglobulin) and Bos d 8 (caseins) were used to detect individuals developing tolerance to cow's milk, whereas Shek and collaborators [24[•]] analyzed the IgE reactivity of 140 cow's milk allergic individuals by using α , β and κ -casein, Bos d 4 and Bos d 5. These authors found that sensitization to caseins was most prevalent, ranging from 47.8% (κ -casein) to 64.6% (α -casein), and that IgE concentrations to caseins were higher than to whey proteins. None of the molecules in the panel was found to be strongly associated milk-induced enterocolitis. Differing

from the above reported studies, it is possible that the use of commercially available proteins of unknown purity and integrity may have influenced the overall study results.

Three molecules from peanuts, Ara h 1, Ara h 2, and Ara h 3, have been used to tentatively define reactivity patterns in peanut-allergic individuals [16^{••}]. Among seven different identified patterns of sensitization to these allergens, monosensitization to a single allergen was relatively rare. The same study group was further characterized using an array of peptides derived from all three allergen sequences in order to detect antibody binding to isolated epitopes (see below for comments on reported results).

Walsh and co-workers [25[•]] reported on 40 patients studied with the widest panel of egg allergens ever used. Although several distinct patterns of reactivity to the different egg white and egg yolk proteins were reported, no clear correlation to clinical symptoms could be observed.

In addition to the 'true' CRD studies discussed above, some recent investigations suggested new diagnostic applications for well characterized allergen molecules. For example, Asero *et al.* [44] hypothesized on the relevance of peach LTP (Pru p 3) as a general marker of allergy against plant-derived food. Studying 40 patients with LTP reactivity, they found a correlation between specific IgE levels to Pru p 3 and spread of clinical reactivity to an increasing number of plant-derived foods. Similarly, the use of bovine serum albumin (Bos d 6) has been suggested as a marker for potential clinical reactivity to mammal's meat [45]. Bolhaar and co-workers [46] described cases of severe reactions to Sharon fruit and identified homologues of Bet v 1 and Bet v 2 as main IgE-binding molecules in Sharon fruit extract. Results of mediator release experiments conducted with Bet v 1 and Bet v 2 indirectly led to the conclusion that Bet v 1-related structures, but not profilins, were biologically active allergens in Sharon fruit and caused the clinical reactivity to this food [46].

Component-resolved diagnostic studies in food allergy using allergen peptides

IgE antibodies recognizing linear epitopes can be studied using short synthetic peptides derived from the allergen amino acid sequences. In a few studies reporting on IgE antibody reactivity against defined epitopes of food allergens, selections of peptides previously defined as allergenic have been tested on selected patient populations in search of a correlation between IgE reactivity and clinical reactivity.

Beyer and coworkers [14] tested decapeptides from α s1-, α s2-, and κ -caseins coupled to ImmunoCAP solid phase (Phadia, Uppsala, Sweden). Consistent with their

previous observations, the authors reported that reactivity to some of these peptides was present only in children with persistent cow's milk allergy, but not in children with transient cow's milk allergy.

The utility of allergen peptides is further exemplified by the study of Matsuo and co-workers on wheat allergy [47^{••}]. In their study, the IgE antibody binding to epitope peptides from ω -5 gliadin and HMW-glutenin showed a promising association to wheat-dependent exercise-induced anaphylaxis.

Peptides from the peanut allergens Ara h 1, Ara h 2 and Ara h 3 have been used to define reactivity patterns at the epitope level in peanut-allergic individuals [16^{••}]. This study led to the identification of a new important epitope on Ara h 1 and revealed a high degree of patient heterogeneity with respect to epitope recognition patterns. The authors suggested that specific patterns of epitope recognition might provide relevant prognostic information regarding persistence of peanut allergy.

Newly identified food allergens suitable for food allergy component-resolved diagnostics

A number of new food allergens have been reported during the past 2 years. These include LTPs from lemon (Cit l 3) and orange (Cit s 3) [48], a germin-like glycoprotein from orange (Cit s 1) [49], oleosin [50[•]], a vicilin-like allergen (Cor a 11) [51] and a LTP (Cor a 8) [52[•]] from hazelnut, a novel plant pathogenesis-related protein from melon (Cuc m 3) [53[•]], a new potential LTP in cauliflower [54], a Bet v 1 homologue in mungbean (Vig r 1) [55], a class III chitinase from indian jujube (Ziz m 1) [56], five new tropomyosins from crustaceans and other invertebrates [57], a 10 kDa buckwheat allergen [58], a recombinant form of Sin a 1, a 2S albumin from mustard [59], a 2S albumin from cashew nut (Ana o 3) [60[•]], seven new parvalbumins (major fish allergens) [61], profilins from tomato (Lyc e 1) [62], melon (Cuc m 2) [63] and orange (Cit s 2) [64] and a recombinant form of LTP from apple (Mal d 3) [65]. Additionally, immunoblot analyses performed in two case studies indicated the presence in latex [66] and carrot [67], respectively, of so far unknown allergens relevant to severe food-allergic reactions.

Although the studies reporting these allergens were not primarily focused on diagnostic applications, they will enable the generation of novel reagents which may be used for food allergy CRD in the future.

Conclusion

Specific IgE antibody testing in food allergy is about to enter into a phase of fundamental change. Most importantly, research in the area of allergenic proteins from a wide range of different sources, their purification, cloning and biotechnical production in a pure

and immunoreactive form has enabled the use of allergen reagents that are no longer limited by the nature and composition of natural extracts. Pure allergen components are becoming increasingly available and can be used to map, characterize and exploit the clinical significance of particular IgE reactivity profiles. This development is expected to bring about more informative ways of analyzing specific IgE responses in different food allergies which, successively, along with more sophisticated approaches to interpret such data, will enable higher diagnostic, predictive and prognostic power of the testing methods in clinical routine.

In order to realize the full potential of these new developments in food allergen science, a new area of evidence-based medicine in food allergy needs to be established. This is indeed a massive undertaking which will require a diversity of carefully designed and performed clinical studies, including all relevant geographical regions, population segments and patient groups.

Another line of recent development, which may become of great help in the clinical exploration of food allergy CRD, is that of protein microarray assays. While specific IgE assays have been designed predominantly as single allergen tests, protein array technologies allow the simultaneous measurement of IgE antibodies of many specificities using complex arrays comprising large numbers of allergens [68–72]. Major screening projects with numerous analytes, which would be very costly and difficult to conduct using conventional technology, may become fully feasible with the availability of such multiplex assay tools [73–79].

While clinical applications drawing from these areas of technological development are still in their infancy, it is likely that they will have significant impact on the design and performance of specific IgE testing in the future.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 246–247).

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In this study a previous report showing birch pollen allergic individuals experiencing allergic reactions to soybean is confirmed by DBPCFC and a risk estimation performed by a limited telephone survey in individuals with birch pollen allergy. Despite the fact that only one purified allergen molecule – the soy allergen Gly m 4 – was employed, a drastic increase in diagnostic sensitivity was noted when the molecule was used for IgE detection, most likely because the allergen has a low abundance in commercial soy extracts.

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In this longitudinal study, 66 cow's milk allergic infants were monitored at 6-month or 1-year intervals for skin reactivity and specific IgE to cow's milk extract, casein, α -lactalbumin and β -lactoglobulin. Development of tolerance to milk was examined by open food challenge. Sixty-eight percent of individuals developed tolerance to milk during the study period and median concentrations of specific IgE of individuals in this group remained similar over time whereas they rose to significantly higher levels in the group with persistent milk allergy. The authors concluded that, provided patient age is considered, levels of specific IgE to milk and casein can be used to predict persistent clinical reactivity to milk.

- 44 Asero R, Mistrello G, Roncarolo D, *et al.* Relationship between peach lipid transfer protein specific IgE levels and hypersensitivity to non-Rosaceae vegetable foods in patients allergic to lipid transfer protein. *Ann Allergy Asthma Immunol* 2004; 92:268–272.

- 45 Fuentes Aparicio V, Sanchez Marcen I, Perez Montero A, *et al.* Allergy to mammal's meat in adult life: immunologic and follow-up study. *J Investig Allergol Clin Immunol* 2005; 15:228–231.

- 46 Bolhaar S, van Ree R, Ma Y, *et al.* Severe allergy to Sharon fruit caused by birch pollen. *Int Arch Allergy Immunol* 2005; 136:45–52.

- 47 Matsuo H, Kohno K, Niihara H, *et al.* Specific IgE determination to epitope peptides of omega-5 gliadin and high molecular weight glutenin subunit is a useful tool for diagnosis of wheat-dependent exercise-induced anaphylaxis. *J Immunol* 2005; 175:8116–8122.

Omega-5 gliadin was shown to be an important allergen in wheat-dependent exercise-induced anaphylaxis (WDEIA). The authors used three synthetic peptides derived from gliadin sequences to study IgE binding in wheat-sensitized individuals and nonatopic controls. It was found that this epitope assay provided a high diagnostic sensitivity and specificity and allowed one to distinguish WDEIA patients from wheat-sensitized individuals with atopic dermatitis.

- 48 Ahrazem O, Ibanez MD, Lopez-Torres G, *et al.* Lipid transfer proteins and allergy to oranges. *Int Arch Allergy Immunol* 2005; 137:201–210.

- 49 Ahrazem O, Ibanez MD, Lopez-Torres G, *et al.* Orange germin-like glycoprotein cit s 1: an equivocal allergen. *Int Arch Allergy Immunol* 2006; 139:96–103.

- 50 Akkerdaas JH, Schocker F, Vieths S, *et al.* Cloning of oleosin, a putative new hazelnut allergen, using a hazelnut cDNA library. *Mol Nutr Food Res* 2005; 50:18–23.

The hydrophobic protein oleosin has been reported to be a relevant allergen in peanut. In this investigation, the authors provide preliminary evidence that oleosin could also represent an allergen in hazelnut and may be a relevant addition to the panel of allergens already available for CRD of hazelnut allergy.

- 51 Lauer I, Foetisch K, Kolarich D, *et al.* Hazelnut (*Corylus avellana*) vicilin Cor a 11: molecular characterization of a glycoprotein and its allergenic activity. *Biochem J* 2004; 383:327–334.

- 52 Schocker F, Lat D, Scheurer S, *et al.* Recombinant lipid transfer protein Cor a 8 from hazelnut: a new tool for in vitro diagnosis of potentially severe hazelnut allergy. *J Allergy Clin Immunol* 2004; 113:141–147.

While the pollen-related allergen Cor a 1.04 has been shown to be the dominant major allergen of hazelnut in areas with birch trees and cause mainly mild reactions, this paper provides evidence that LTP may be used as a marker for more severe forms of food allergy in the Mediterranean area. Since 70% of the patients described had systemic reactions to hazelnut, Cor a 8 has to be considered as an important molecule for CRD of hazelnut allergy.

- 53 Asensio T, Crespo JF, Sanchez-Monge R, *et al.* Novel plant pathogenesis-related protein family involved in food allergy. *J Allergy Clin Immunol* 2004; 114:896–899.

This paper reports the purification of a new 16 kDa allergen, Cuc m 3, from melon, with sequence homology to members of the PR-1 protein family. No member of this family has previously been reported as an allergen. Twelve of 17 melon-allergic individuals showed IgE binding to purified Cuc m 3, indicating that PR-1 proteins may represent interesting new target compounds for CRD studies in plant food allergy.

- 54 Hernandez E, Quirce S, Villalba M, *et al.* Anaphylaxis caused by cauliflower. *J Investig Allergol Clin Immunol* 2005; 15:158–159.

- 55 Mittag D, Vieths S, Vogel L, *et al.* Birch pollen-related food allergy to legumes: identification and characterization of the Bet v 1 homologue in mungbean (*Vigna radiata*), Vig r 1. *Clin Exp Allergy* 2005; 35:1049–1055.

- 56 Lee MF, Hwang GY, Chen YH, *et al.* Molecular cloning of Indian jujube (*Zizyphus mauritiana*) allergen Ziz m 1 with sequence similarity to plant class III chitinases. *Mol Immunol* 2006; 43:1144–1151.
 - 57 Marknell DeWitt Å, Mattsson L, Lauer I, *et al.* Recombinant tropomyosin from *Penaeus aztecus* (rPen a 1) for measurement of specific immunoglobulin E antibodies relevant in food allergy to crustaceans and other invertebrates. *Mol Nutr Food Res* 2004; 48:370–379.
 - 58 Matsumoto R, Fujino K, Nagata Y, *et al.* Molecular characterization of a 10-kDa buckwheat molecule reactive to allergic patients' IgE. *Allergy* 2004; 59:533–538.
 - 59 Palomares O, Cuesta-Herranz J, Rodriguez R, *et al.* A recombinant precursor of the mustard allergen Sin a 1 retains the biochemical and immunological features of the heterodimeric native protein. *Int Arch Allergy Immunol* 2005; 137:18–26.
 - 60 Robotham JM, Wang F, Seamon V, *et al.* Ana o 3, an important cashew nut (*Anacardium occidentale* L.) allergen of the 2S albumin family. *J Allergy Clin Immunol* 2005; 115:1284–1290.
- This paper describes the cloning and characterization of a new major allergen, Ana o 3 (2S albumin), from cashew nut. Isoforms of the corresponding native protein were purified and analysed by mass spectrometry. Among 26 individuals with self-reported reactions to cashew nut, 21 showed serum IgE reactivity to recombinant Ana o 3 in immunoblot analysis. Several linear IgE-binding epitopes were identified by the use of an array of overlapping peptides derived from the Ana o 3 sequence. Like other 2S albumins, the protein may represent another marker molecule for potentially severe food allergic reactions.
- 61 van Do T, Elsayed S, Florvaag E, *et al.* Allergy to fish parvalbumins: studies on the cross-reactivity of allergens from 9 commonly consumed fish. *J Allergy Clin Immunol* 2005; 116:1314–1320.
 - 62 Westphal S, Kempf W, Foetisch K, *et al.* Tomato profilin Lyc e 1: IgE cross-reactivity and allergenic potency. *Allergy* 2004; 59:526–532.
 - 63 Lopez-Torreon G, Crespo JF, Sanchez-Monge R, *et al.* Allergenic reactivity of the melon profilin Cuc m 2 and its identification as major allergen. *Clin Exp Allergy* 2005; 35:1065–1072.
 - 64 Lopez-Torreon G, Ibanez MD, Ahrazem O, *et al.* Isolation, cloning and allergenic reactivity of natural profilin Cit s 2, a major orange allergen. *Allergy* 2005; 60:1424–1429.
 - 65 Zuidmeer L, van Leeuwen WA, Budde IK, *et al.* Lipid transfer proteins from fruit: cloning, expression and quantification. *Int Arch Allergy Immunol* 2005; 137:273–281.
 - 66 Asero R, Mistrello G, Roncarolo D, *et al.* Detection of novel latex allergens associated with clinically relevant allergy to plant-derived foods. *J Allergy Clin Immunol* 2005; 115:1312–1314.
 - 67 Fernandez-Rivas M, Gonzalez-Mancebo E, van Leeuwen WA, *et al.* Anaphylaxis to raw carrot not linked to pollen allergy. *Allergy* 2004; 59:1239–1240.
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 - 78 Harwanegg C, Laffer S, Hiller R, *et al.* Microarrayed recombinant allergens for diagnosis of allergy. *Clin Exp Allergy* 2003; 33:7–13.
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