REVIEW ARTICLE

Use of allergen components begins a new era in pediatric allergology

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Abstract

Molecular allergology is a breakthrough science that enables the quantification of IgE and IgG antibodies against individual allergen protein components at a molecular level.

The diagnosis of IgE-mediated allergic disorder among children is based on clinical history and sensitization demonstrated through an allergy test. Identifying whether the sensitization is primary (species specific) or a result of cross-reactivity to proteins with similar protein structures helps the clinician to judge the risk of allergic reaction. This is possible today because allergen component tests are now available for clinicians to use in everyday practice.

This article focuses on clinical utility through the prediction of cross-reactivity or primary sensitization, estimation of the risk of reaction to heated food and the risk of severe clinical symptoms.

How IgE-mediated allergy is diagnosed

The diagnosis of IgE-mediated allergic disorders is based on the clinical history and sensitization demonstrated through an allergy test. Allergen-specific IgE is detected with *in vitro* and/or *in vivo* testing. In some cases, allergen provocation tests are performed to confirm an allergy diagnosis.

Specific IgE is currently determined using allergen extracts as test allergens – a situation that gives rise to two types of problems (1, 2). The first is the difficulty of standardizing the allergens used as substrates. These extracts may differ in terms of their allergenic content owing to the natural variability of the allergen source.

The second problem is that the tests used are not capable of differentiating between primary sensitization and immunological cross-reactivity, which in some cases entails a significant risk of serious symptoms. This, together with the increased prevalence in childhood food allergy (3), causes difficulties for clinicians in their day-to-day work of interpreting the results of the allergy tests.

The limitations described earlier have led to the introduction of intensive research activity in molecular allergology. The term Component Resolved Diagnostics was introduced by Valenta et al. (4). However, the isolation and characterization of allergen components began far earlier. One of the first food allergen component to be described, Gad c 1 from cod, had already been purified by Aas and Sayed (5) in the late sixties in Norway.

It is important to understand some basics of molecular allergology in order to understand how the tests can be used clinically. Almost anything containing proteins can be an allergen source. Each source contains many different proteins, some of which can cause allergy. Each allergen component commonly has several different epitopes. An epitope is the actual three-dimensional binding site for an antibody. Knowledge of the protein structure, the protein families, and the stability during heating and digestion enables the use of allergen components in the clinic to be optimized.

These differences in stability explain why some food allergens may be tolerated when raw while others require

cooking. Also, some allergens cause clinical reactions ranging from mild to moderate and severe, whereas others will cause sensitization without any clinical reaction.

The allergen components are named after their Latin family name. Ara h 1 stands for allergen 1 from *Arachis hypogea* (peanut).

Some allergen components are unique markers for a specific allergen source. The value of identifying these species-specific allergen components lies in being able to narrow down the primary sensitizer that causes certain reactions to just one specific source, e.g., cat.

Identifying whether the sensitization is primary (species specific) or a result of cross-reactivity to proteins with similar protein structures makes it easier for the clinician to judge the risk of reaction on exposure to different allergen sources.

The development of allergen components in pure form has made it possible to resolve many of these problems. In terms of production techniques, they can be either produced biotechnologically in recombinant form or purified from their original sources. In other words, the main area of application for purified natural or recombinant allergen components is in the precise identification of the allergies that cause the disease (2). Many allergen sources have not yet been fully characterized, and for the foreseeable future, allergen extracts will be needed for the diagnosis of unusual allergies and in the cases of unusual sensitization patterns to common allergen sources. The different methods must complement each other.

The allergen components are available for clinicians and are used in accordance with the same techniques and blood sampling as for the usual ImmunoCAP® (Phadia AB, Uppsala, Sweden) tests.

Allergen components are also available on an ImmunoCAP® ISAC biochip.

The allergen components are useful not only in diagnosis and the estimation of risk but also in the standardization of immunotherapy extracts (6). In this way, the content of each relevant allergen component can be determined. In the near future, it will therefore be possible to produce inherently standardized immunotherapy extracts, containing only relevant allergenic protein in defined and constant proportions. This latter aspect will not be discussed in this article.

Typical example of how allergen components meet clinical needs

On investigation, a child with a suspected peanut allergy gives a positive skin prick test or *in vitro* allergy test for peanut extract. The prognosis can be very different depending on whether the sensitization is linked to a Bet v 1-like protein, a seed storage protein, or an lipid-transfer protein (LTP). In the first case, there is almost no risk of the child's experiencing serious anaphylactic shock. In the second and third cases, the child is advised to carry injectable adrenalin (e.g., Epipen™, Anapen™, or Jext™). This leads us to the discussion of how the different allergen components should be used and interpreted in the clinic.

Clinical usefulness in the investigation into food allergies

Peanuts

Peanuts are the most common food associated with fatal allergic reactions in the Western world (7). The prevalence seems also to increase in Asia, and peanut allergy now ranks in top ten food stuff causing food allergy (8). Accidental ingestion of peanut may cause severe allergic reactions in susceptible individuals.

The prevalence of peanut allergy has increased and has been estimated to be as high as 2% in some regions. The symptoms following the ingestion of peanut can vary from mild reactions, such as oral allergy syndrome (OAS), to respiratory distress and severe systemic reactions needing medical care, such as anaphylactic shock. Unfortunately, peanut sensitization, established by allergen-specific IgE analysis of blood samples or skin prick testing against peanut extract, has a low positive predictive value because many sensitized individuals are tolerant to peanut. The reason for this lack of precision is cross-reactive IgE antibodies with low clinical significance. Examples of this are IgE antibodies induced by PR-10 protein in pollen that cross-react with their peanut homologues, profilins, or cross-reactive carbohydrate determinants. This means that sensitization to peanut is quite common in a general population. Nicolaou et al. (9) have investigated the prevalence of peanut sensitization in an unselected population-based cohort in Manchester (MAAS, n = 1085). They found that 12% of the 8-yr-old children were sensitized. Only 24% of the children sensitized to peanut demonstrated a clinical peanut allergy on the basis of food challenge results.

Five peanut components are clinically relevant and available to clinicians. To date, 13 peanut allergens have been detected.

The peanut seed storage proteins Ara h 1, Ara h 2, and Ara h 3 are all major allergens and seem to be associated with primary sensitization to peanut in susceptible individuals. Among these seed storage proteins, Ara h 2 in particular is considered a risk marker for severe allergic reactions. In individuals sensitized to peanut and with a cutoff point of 0.35 kU/L, Ara h 2 correctly classified 97.5% of the patients (10). Importantly, all children with peanut allergy were given correct classification. Sensitization to multiple allergens is a stronger indication of more severe reactions than sensitization to only one of the components (11).

Ara h 8 is a PR10 protein, a Bet v 1 homologue, and thus a marker for primary sensitization to pollen from birch and alder.

IgE against Ara h 9 is often associated with systemic and more severe reactions in addition to OAS, especially in southern Europe (12). Ara h 9 is a LTP, and in most of the cases, sensitization is probably due to primary sensitization to peach or other LTP-containing fruits (12). Vereda et al. (13) have shown the geographical differences regarding the peanut allergen component sensitization pattern between Spain, Sweden and the United States.

Egg

Egg white is the most important source of allergens in egg and contains 23 different proteins (14). The most important allergens that have been identified and for which the clinician can test are ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin/conalbumin (Gal d 3), and lysozyme (Gal d 4) (15).

Although ovomucoid comprises only 10% of the total egg white protein, it has been shown to be the dominant allergen. Ovomucoid has several unique characteristics, such as stability to heating and breaking down by proteinases. It also appears to be allergenic in minute quantities, and testing for ovomucoid has proven helpful in the prognosis and diagnosis of egg allergy.

High concentrations of ovomucoid-specific IgE are associated with persistent egg allergy (15).

When an individual allergic to egg starts to develop tolerance to hen's egg, he or she first becomes tolerant to heated egg and subsequently to raw egg, which has been described in several case reports (16).

Differences in IgE antibodies against ovomucoid were found in patients depending on the reactivity to raw and cooked egg (17). Low levels of IgE antibodies against ovomucoid were associated with tolerance to cooked egg. Furthermore, the quantification of ovomucoid antibodies can guide the clinician's decision on whether to perform a challenge. Ando et al. (17) show that a concentration of IgE antibodies against ovomucoid higher than *c*. 11 kUA/l (positive decision point) indicates a high risk of reacting to heated (as well as raw) egg. At the same time, a concentration below *c*. 1 kUA/l (negative decision point) means that there is a low risk of reaction to heated egg, although the patient may well react to raw egg.

Benhamou et al. (18) have found differences in egg-specific IgE levels for patients with severe, moderate, or absent reactions at challenge, highest for patients with severe reactions and decreasing with the severity of reaction. This kind of differences regarding levels for ovomucoid and severity in challenge are yet to be described.

Milk

The majority of patients allergic to milk are sensitized to several cow's milk proteins. However, the profile of the IgE response to these components varies greatly.

The most important allergens in milk are caseins (Bos d 8), beta-lactoglobulins (Bos d 5), and alpha-lactoglobulins (Bos d 4), although allergies to other minor proteins such as bovine serum albumins (Bos d 6) have also been reported (19).

There is now growing evidence that casein seems to be a major allergen component to test for in the treatment of a patient with cow's milk allergy.

Garcia-Ara and co-workers have been following children with cow's milk allergy. They have observed that casein is the protein that best discriminates between persistent and transient allergy (20). The same Spanish group has also been studying reactions to accidental exposure to milk in children with cow's milk allergy. They found that it was relatively common and that 15% of the group had severe reactions. The risk factors for such reactions include high levels of IgE against cow's milk and casein in combination with asthma.

Gern and Sampson have studied allergic reactions in patients with cow's milk allergy who eat so-called non-dairy products (21). They found that casein was often the cause of the reaction. Casein is used as an extender in sausages, soups, and stews.

D'Urbano et al. (22) showed that in patients with a positive food challenge to milk, casein (Bos d 8) was the milk allergen component against which they most frequently had IgE.

Wheat

Wheat allergy is common worldwide (8, 23) and is sometimes difficult to diagnose for the pediatric allergist. Part of the reason is that a positive result to wheat flour extract may not always correlate with clinical symptoms (24), which indicates that *in vitro* diagnosis of allergy to wheat may be improved by using wheat allergen components. There are a number of strong candidates among the wheat components currently undergoing clinical evaluation. They will most likely improve the management of patients allergic to wheat.

Wheat commonly cross-reacts with grass pollen, which causes a problem with overdiagnosis of wheat allergy. The typical situation is that the clinician performs a skin prick test for wheat or orders a wheat-specific IgE Ab measurement for a patient allergic to grass. Owing to cross-reaction, this test will most probably be positive. The clinician might interpret this as an indication of wheat allergy and incorrectly advise the patient to avoid wheat in the diet. Improved species-specific diagnostics for wheat are obviously needed.

To date, we can test for one major wheat component when investigating suspected hypersensitivity reactions to wheat in children and adults.

In children, IgE antibodies against omega-5 gliadin (Tri a 19) are associated with a risk of IgE-mediated reactions to wheat (25, 26). It has been suggested that the level of antibodies against omega-5 gliadin acts as a marker for clinical reactivity and an aid when deciding whether to perform a wheat challenge (26). IgE antibodies against Tri a 19 in adults are linked to a risk of exercise-induced reactions associated with the ingestion of wheat (27). LTP to wheat seems also to be a clinical-relevant component when investigating wheat hypersensitivity but is not yet available for clinical use.

Fish

Fish proteins are sometimes responsible for life-threatening IgE-mediated allergic reactions.

Fish parvalbumins from cod (*Gadus morhua*) Gad c 1 and carp (*Cyprinus carpio*) Cyp c 1 are both major fish parvalbumin proteins and representative markers for fish sensitization in general (5). The different content of parvalbumin in species like cod, whiff, or swordfish may explain tolerance to some

species (28). The extensive cross-reactivity between parvalbumins from different species means that Gad c 1 and Cyp c 1 are valuable tools in diagnosing patients with fish allergy. Both have remarkable stability, which may explain why sensitization can result because of ingestion even after cooking, via contact with and inhalation of vapor from cooking. Sensitization to a fish parvalbumin suggests caution in the administration of all fish species to reactive patients (29).

Soy

Soybean allergy in children is known to be mediated primarily by contact with allergen via the gastrointestinal tract, often in the form of soya-based milk substitute products, particularly in infants allergic to cow's milk. The primary sensitizers seem to be the most important soya proteins Gly m 5 and Gly m 6 (30, 31). Soy allergy may also be acquired following primary sensitization to birch pollen, owing to IgE cross-reactivity between the most important birch pollen allergen Bet v 1, and its homologous protein in soybean, Gly m 4 (32). To date, pollen-mediated soy allergy has been mainly described in adults. This type of soy allergy seems also to be a problem among the pediatric population, and there have recently been reported four children allergic to birch pollen who experienced severe allergic reactions following the ingestion of soy milk during the pollen season (33).

Gly m 4 has been shown to be a risk factor for severe OAS or systemic reactions to soya in patients allergic to birch pollen (32). Gly m 4 is also cross-reactive with Ara h 8, and in Europe, approximately two-thirds of patients allergic to soya are allergic to peanut. Targeted diagnostic testing with Gly m 4 is recommended in pollen-sensitized patients where allergy to soya is suspected, especially if the soya extract test result is negative. Some patients sensitized to Gly m 4 can show low or even negative IgE results with soya extract because of a low Gly m 4 content in the extract (33).

Furred animals

Cross-reactions also occur between our most common domestic animals, such as cats, dogs, and horses. This is partly new knowledge and might explain why so many of our patients allergic to furred animals are often sensitized to more than one species. In the German MAS (Multicentre Allergy Study) cohort, Matricardi and co-workers identified 56 children sensitized to cat at the age of 10. Fifty-seven per cent of them reported having concomitant allergic sensitization to dog. Forty-one children were sensitized to dog, and 73% were also sensitized to cat (34). Liccardi and co-workers identified 35 adults sensitized to horse, of whom 23 were reported to have concomitant allergic sensitization to dog and 25 to cat (35). Baatenburg de Jong et al. have recently shown that among a group of 776 polysensitized children, 87% were sensitized to dog and 74% were sensitized to cat (36). These studies indicate either a strong comorbidity between furry animal allergies or prevalent cross-reactions or a combination of both. Challenge with animal dander is theoretically possible and would reveal the actual reactions to each dander. However, this is not a method commonly used today because of the risk of severe reactions. These conditions can be now studied through allergen component testing.

Fel d 1 is the most important allergen component in cat (1), which indicates primary sensitization. Up to 90% of patients allergic to cat have IgE antibodies against Fel d 1. This allergen component can be used as a specific marker, which indicates that immunotherapy treatment of cat is probably of clinical value. Among individuals allergic to cat, Grönlund and co-workers found higher levels of IgE against Fel d 1 in children with asthma compared with children with allergic rhinoconjunctivitis (37). This indicates that Fel d 1 could be used as a marker for an increased risk of lower respiratory disease among cat-sensitized individuals. Other cat components available for testing are Fel d 2 and Fel d 4.

IgE against cat serum albumin Fel d 2 is likely to cross-react with most other mammalian albumins, such as dog Can f 3, horse Ecu c 3, pig Sus s PSA (pig serum albumin), and cow Bos d 6. It can also cause reactions following the ingestion of pork (the cat-pork syndrome); about 15–40% of patients allergic to cat have IgE against Fel d 2 (1).

The picture for primary sensitization to dog is more complex.

To date, Can f 1, Can f 2, and Can f 5 have been found to be specific allergen components that indicate primary sensitization; *c.* 50–90%, 20–33%, and 70% of patients allergic to dog have IgE antibodies against Can f 1, 2, and 5, respectively (1). A completely new finding is the recent identification of Can f 4 as another species-specific allergen component for dog (38). This complexity might explain why dog allergy can, in some cases, present clinical difficulties. It is quite common for a patient allergic to dog to tell the clinician that he/she can tolerate some dogs but reacts to contact with others. Future research will clarify whether the composition of the dog allergen components differs between various breeds of dog, which would explain the clinical picture.

Equ c 1, a lipocalin, is considered the major allergen in horse dander. New data have been presented but not yet published that Equ c 1 cross-reacts with Fel d 4, which belongs to the same protein family. This new knowledge and insight means that we may be overdiagnosing horse allergy at present. It may be that patients are only sensitized to cat, but our interpretation may be that they are also allergic to horse, and vice versa. We now have the tools to understand the underlying mechanisms behind sensitization in more detail. Consequently, we should be more careful in advising on avoiding animal dander before we know the primary sensitizer.

Allergen components on microarrays

The term 'microarray or biochip' refers to the distribution of small amounts of biomolecules on a surface in a regular, compact pattern. In contrast to conventional diagnosis, microarrays allow us to investigate IgE reactivity against a large number of different allergenic components with a single, rapid test.

The amount of patient serum required is far smaller than in conventional immunoassay. In fact, as little as 20 μl is enough to determine IgEs against hundreds of individual allergens, while conventional tests require 50 μl for each allergen tested.

This facilitates the use of the technique in pediatric patients, because such a minute amount of serum can be obtained from a simple blood sample.

The first experimental microarray system for allergy diagnosis was reported in 2000 (39). Later on, microarrays were developed with a growing number of recombinant and purified molecules. The ISAC prototype commercialized by VBC Genomics/Phadia was the first protein microarray applied to the detection of sIgE (40). There are a number of studies (41–43) validating microarray technology using homework or commercially available technology (ImmunoCAP® ISAC).

ISAC is an IgE antibody assay specifically designed to help clinicians identify the presence and quantify the amount of cross-reactive IgE antibodies among the different food and pollen allergen groups that are known to share extensive homology (44). The microarray generates a fluorescent image, which is analyzed by special software that calculates the IgE results semiquantitatively for each allergenic component. IgE concentration is measured in arbitrary units termed ISAC Standardized Units (ISUs), and these values are divided into four classes (negative, low, intermediate, and high).

Interpreting 112 allergen component test results per patient is challenging for the clinician. Soon, microarray technology will be combined with PC-based intelligent support for interpretation. Clinical trials have been performed, and preliminary data indicate that an interpretation tool helps the practicing allergy specialist assimilate and interpret the vast amount of IgE antibody data from the chip-based microarray assay. This should make the issue of food cross-reactivity more manageable for the practicing clinician.

Could molecular allergology replace the food challenge?

The measurement of allergen components has the potential to reduce the number of food challenge. The reason why food challenge not yet can be replaced is that not all allergic sources in the various foods have yet been completely characterized and evaluated. From a health economic perspective, the health service would save money and reduce the risks if allergen components were used instead of food challenges. We therefore need a method even safer and better than the challenges we use today. The double-blind placebo-controlled food challenge (DBPCFC) has long been the standard in the diagnosis of food allergy as a benchmark test from which to judge the diagnostic characteristics of the clinical history, skin prick test, and IgE antibody serology. The drawback is that open challenge can give false-positive results ranging from 20% to 71% (44). However, positive placebo reactions that may occur during the DBPCFC can be as high as 35%. False-negative open challenges occur in 1–3% of cases.

Furthermore, the problem today is that too few patients are offered or are prepared to undergo a challenge, owing to scarce resources and the risk of severe reactions. Especially, nut allergies are difficult to food challenge, and as a consequence, few patients with suspected nut or peanut allergies undergo challenges. There is therefore a great need for improved diagnosis, in which the testing of allergen components can be very helpful. Zijlstra and co-workers have performed food challenges on this group, finding that 58% of the individuals had unnecessarily avoided hazelnuts and 33% peanuts (45).

D′Urbano et al. (22) have investigated children with suspected cow's milk allergy, comparing milk allergen components with milk challenges. The results indicate that serial testing of IgE against milk and microarray ImmunoCAP® ISAC have a clinical performance very close to that of the food challenge. Using this two-step approach, the clinician would have detected that 27 of 29 children should have a milk-free diet. Using only IgE against cow's milk at the pediatric clinic in primary health care would have eliminated the need for a challenge in about 27% of the patients.

This sequential use of the two tests would have led to a 50% reduction in the number of challenges.

Even more remarkable is that this reduced the number of positive challenges to five compared with the previous 17. These data are very promising with regard to reducing risks for children with allergies.

According to D′Urbano et al., serial use of the two tests could be considered from the point of view of clinical application, based on the opportunity that:

1 Pediatricians in outpatient care or general practitioners in primary health care identify patients with a high probability of allergy, based on case history and detection of IgE against cow's milk. The children with high probability of milk allergy are referred to secondary care.

2 The referred patients are screened with the microarray to assess whether a food challenge should be carried out in secondary- or tertiary-level health care.

Clinical advantages of microarrays

Allergic patients with a complex symptomatology, such as severe eczema, unstable asthma, and chronic urticaria, are especially suitable for the investigation of IgE reactivity using microarrays. The number of molecular allergens gives comprehensive and detailed information about the patient's sensitization profile.

To illustrate the clinical advantages of microarray, we show two pediatric cases from a birth cohort with high risk of developing allergies, and blood samples were taken at the ages of 6 and 18 months and at 6 and 18 yrs (46, 47). In this study, sera from these four sampling occasions were analyzed retrospectively with a component-resolved *in vitro* diagnosis technique using the ImmunoCAP® ISAC microarray assay.

The IgE antibody assay results were compared with each patient's clinical history. Two cases with severe allergic and asthmatic disease are described below to demonstrate the value of component-resolved diagnostics.

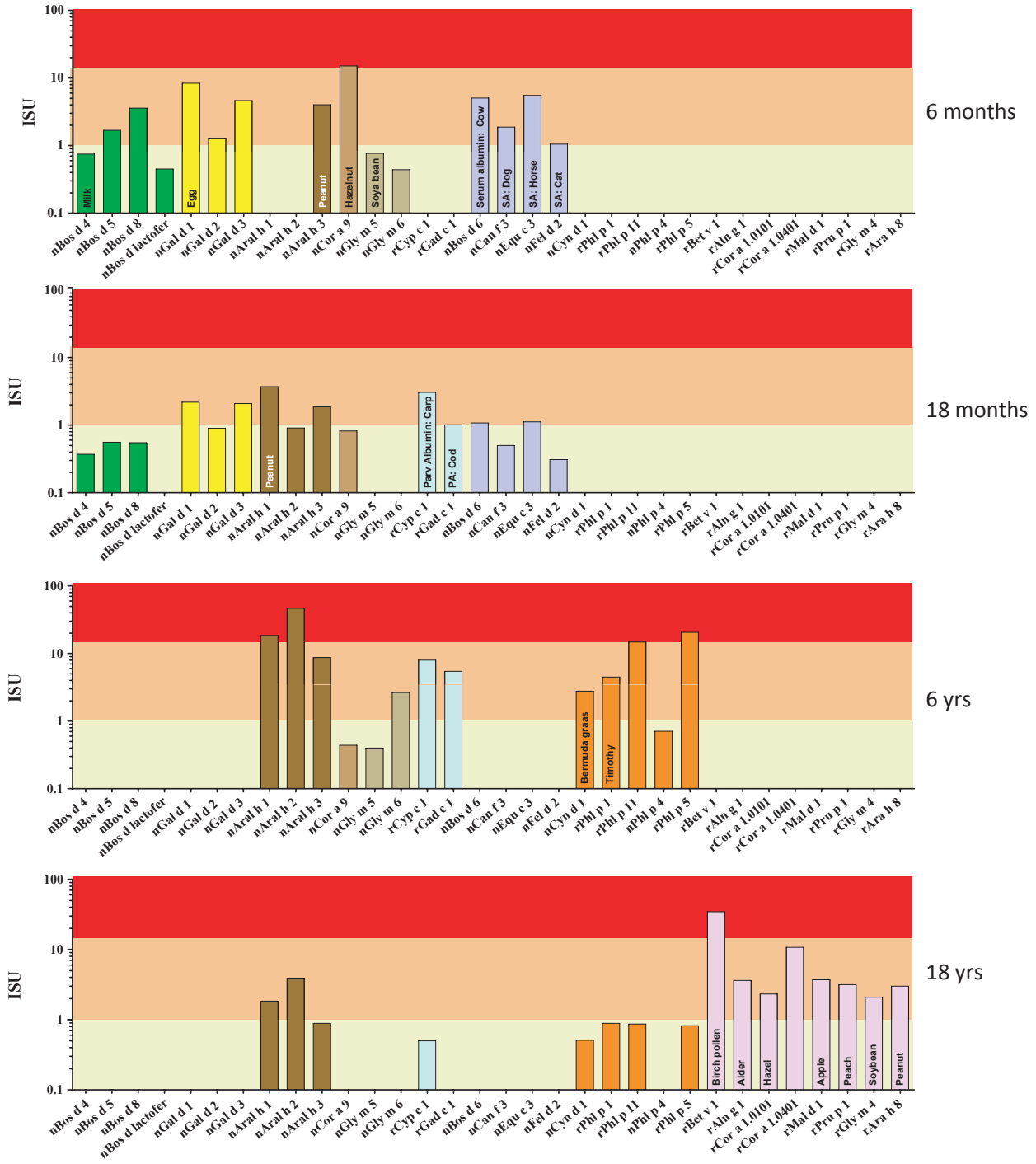

Figure 1 Test results from case number 1. The presence of IgE antibodies against several egg white and milk components confirms the egg and milk allergy in early childhood. In parallel, there were also positive values at an early age for hazelnut (Cor a 9), peanut (Ara h 1, Ara h 2, Ara h 3) and soya (Gly m 5, Gly m 6). The values for fish (Cyp c 1 and Gad c 1) peaked at 6 years of age, in accordance with the medical history. The value of the timothy pollen component (Phl p 5) was high at 6 years of age, but relatively low at 18 years. In contrast, the birch component (Bet v 1) was very high at 18 years of age, but negative at 6 years, also in accordance with the clinical history.

Case 1

A boy with two allergic parents had developed severe atopic eczema and food allergy at the early age of 2 months. Milk, egg, and fish were diagnosed as the offending allergens and were avoided. Soy supplement was used because of an inadequate supply of breast milk. He also had wheezing problems at an early age and was diagnosed with asthma at 6 months of age. Allergic rhinitis was diagnosed at the age of 2, and he was sensitized to pollen and furred animals. The eczema disappeared at the age of 10, and his severe asthma became mild at 15 yrs. At 18 yrs, he had allergic rhinitis and mild asthma and was sensitized to birch and timothy.

Component results

The presence of IgE antibodies against several egg white and milk components (Fig. 1) confirms the egg and milk allergies in early childhood. In parallel, there were also positive values at an early age for hazelnut (Cor a 9), peanut (Ara h 1, Ara h 2, and Ara h 3), and soy (Gly m 5 and Gly m 6). The boy subsequently experienced breathing problems when eating nuts and peanuts. Retrospectively, he was most likely allergic to soy because the soy supplement caused colic and the eczema did not fully improve. The values for fish (Cyp c 1 and Gad c 1) peaked at 6 yrs of age, in accordance with the medical history. The value of the timothy pollen component (Phl p 5) was high at 6 yrs of age, but relatively low at 18 yrs. In contrast, the birch component (Bet v 1) was very high at 18 yrs of age, but negative at 6 yrs, also in accordance with the clinical history.

Case 2

A boy with severe atopic eczema and food allergy (vomiting), which started at the age of 3 months, was studied. Egg, fish, and birch pollen were positive in skin prick tests and diagnosed as the offending allergens. In addition, OAS-like symptoms in response to peanut and shellfish were reported at 6 yrs of age, although these reactions were never confirmed by a test. At 18 yrs of age, breathing problems following the ingestion of peanuts were still occurring.

Component results

The presence of IgE antibodies against several egg, fish, and birch pollen components confirms the diagnosed allergies. Furthermore, IgE antibodies against peanut (Ara h 1, 2, and 3) and shellfish (tropomyosin) components are registered before the reactions to peanut and shellfish are reported.

The allergen component results clearly show the progression of allergy for these two children. If the component results had been available at the time of the medical examination, the patients would have been managed differently. Furthermore, a better understanding of the underlying causes of the symptoms would have been possible.

Conclusions

The use of allergen components will pave the way for a more individual approach when we investigate and care for our patients with suspected allergic diseases. Using molecular allergology, we can now already better diagnose, prognose, and grade the allergic diseases. We can also get help with choosing a more individualized treatment and get better support regarding which individuals should be advised to avoid specific allergens.

Conflict of interest

Magnus Borres is medical director at Phadia AB. Philippe Eigenmann has received speakers honoraria from Phadia and ALK.

References

1. Sastre J. Molecular diagnosis in allergy. *Clin Exp Allergy* 2010: **40**: 1442–60.
2. Lucas JM. Microarrays: molecular allergology and nanotechnology for personalised medicine (I). *Allergol Immunopathol* 2010: **38**: 153–61.
3. Prescott S, Allen KJ. Food allergy: riding the second wave of the allergy epidemic. *Pediatr Allergy Immunol* 2011: **22**: 155–60.
4. Valenta R, Lidholm J, Niederberger V, et al. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin Exp Allergy* 1999: **29**: 896–904.
5. Aas K, Elsayed SM. Characterization of a major allergen (cod). Effect of enzymic hydrolysis on the allergenic activity. *J Allergy* 1969: **44**: 333–43.
6. Valenta R, Niederberger V. Recombinant allergens for immunotherapy. *Clin Immunol* 2007: **119**: 826–30.
7. Hourihane JO, Kilburn SA, Dean P, Warner JO. Clinical characteristics of peanut allergy. *Clin Exp Allergy* 1997: **27**: 634–9.
8. Ebisawa M, Ikematsu K, Imai T, Tachimoto H. Food allergy in Japan. *Allergy Clin Immunol Int: J World Allergy Org* 2003: **15**: 214–7.
9. Nicolaou N, Poorafshar M, Murray C, et al. Allergy or tolerance in children sensitized to peanut: prevalence and differentiation using component-resolved diagnostics. *J Allergy Clin Immunol* 2010: **125**: 191–7.
10. Nicolaou N, Murray C, Belgrave D, et al. Quantification of specific IgE to whole peanut extract and peanut components in prediction of peanut allergy. *J Allergy Clin Immunol* 2011: **127**: 684–5.
11. Astier C, Morisset M, Roitel O, et al. Predictive value of skin prick tests using recombinant allergens for diagnosis of peanut allergy. *J Allergy Clin Immunol* 2006: **118**: 250–6.
12. Lauer I, Dueringer N, Pokoj S, et al. The non-specific lipid transfer protein, Ara h 9, is an important allergen in peanut. *Clin Exp Allergy* 2009: **39**: 1427–37.
13. Vereda A, Van Hage M, Ahlstedt S, et al. Peanut allergy: clinical and immunologic differences among patients from 3 different geographic regions. *J Allergy Clin Immunol* 2011: **127**: 603–7.
14. Benhamou AH, Caubert JC, Eigenmann PA, et al. State of the art and new horizons in the diagnosis and management of egg allergy. *Allergy* 2010: **65**: 283–9.
15. Bernhisel-Broadbent J, Dintzis HM, Dintzis RZ, Sampson HA. Allergenicity and antigenicity of chicken egg ovomucoid (Gal d III) compared with ovalbumin (Gal d I) in children with egg allergy and in mice. *J Allergy Clin Immunol* 1994: **93**: 1047–59.
16. Eigenmann PA. Anaphylactic reactions to raw eggs after negative challenges with

cooked eggs. *J Allergy Clin Immunol* 2000: **105**: 587–8.
17. Ando H, Movérare R, Kondo Y, et al. Utility of ovomucoid-specific IgE concentrations in predicting symptomatic egg allergy. *J Allergy Clin Immunol* 2008: **122**: 583–8.
18. Benhamou AH, Zamora SA, Eigenmann PA. Correlation between specific immunoglobulin E levels and the severity of reactions in egg allergic patients. *Pediatr Allergy Immunol* 2008: **19**: 173–9.
19. Wal JM. Bovine milk allergenicity. *Ann Allergy Asthma Immunol* 2004: **93** (Suppl. 3): S2–11.
20. Boyano-Martínez T, García-Ara C, Pedrosa M, Díaz-Pena JM, Quirce S. Accidental allergic reactions in children allergic to cow's milk proteins. *J Allergy Clin Immunol* 2009: **123**: 883–8.
21. Gern JE, Yang E, Evrard HM, Sampson HA. Allergic reactions to milk-contaminated "nondairy" products. *N Engl J Med* 1991: **324**: 976–9.
22. D'Urbano LE, Pellegrino K, Artesani MC, et al. Performance of a component-based allergen-microarray in the diagnosis of cow's milk and hen's egg allergy. *Clin Exp Allergy* 2010: **40**: 1561–70.
23. Palosuo K. Update on wheat hypersensitivity. *Curr Opin Allergy Clin Immunol* 2003: **3**: 205–9.
24. Komata T, Söderström L, Borres MP, Tachimoto H, Ebisawa M. Usefulness of wheat and soybean specific IgE antibody titers for the diagnosis of food allergy. *Allergol Int* 2009: **58**: 599–603.
25. Palosuo K, Varjonen E, Kekki OM, et al. Wheat omega-5 gliadin is a major allergen in children with immediate allergy to ingested wheat. *J Allergy Clin Immunol* 2001: **108**: 634–8.
26. Ito K, Futamura M, Borres MP, et al. IgE antibodies to omega-5 gliadin associate with immediate symptoms on oral wheat challenge in Japanese children. *Allergy* 2008: **63**: 1536–42.
27. Matsuo H, Dahlström J, Tanaka A, et al. Sensitivity and specificity of recombinant omega-5 gliadin-specific IgE measurement for the diagnosis of wheat-dependent exercise-induced anaphylaxis. *Allergy* 2008: **63**: 233–6.
28. Griesmeier U, Vázquez-Cortés S, Bublin M, et al. Expression levels of parvalbumins determine allergenicity of fish species. *Allergy* 2010: **65**: 191–8.
29. Fiocchi A, Nowak-Węgrzyn A. The fascinating world of molecular diagnosis in the management of food allergy: nondum matura est. *Curr Opin Allergy Clin Immunol* 2011: **11**: 200–3.
30. Holzhauser T, Wackermann O, Ballmer-Weber BK, et al. Soybean (Glycine max) allergy in Europe: Gly m (beta-conglycinin) and Gly m 6 (glycinin) are potential diagnostic markers for severe allergic reactions to soy. *J Allergy Clin Immunol* 2009: **123**: 452–8.
31. Ito K, Sjölander S, Movérare R, et al. IgE to Gly m 5 and Gly m 6 is associated with severe allergic reactions to soybean in Japanese children. *J Allergy Clin Immunol* 2011: May 7 [Epub ahead of print]. PMID: 21555150.
32. Mittag D, Vieths S, Vogel L, et al. Soybean allergy in patients allergic to birch pollen: clinical investigation and molecular characterization of allergens. *J Allergy Clin Immunol* 2004: **113**: 148–54.
33. Kosma P, Sjölander S, Landgren E, Borres MP, Hedlin G. Severe reactions after intake of soy drink in birch pollen allergic children sensitized to Gly m 4. *Acta Paediatr* 2011: **100**: 305–6.
34. Matricardi PM, Bockelbrink A, Beyer K, et al. Primary versus secondary immunoglobulin E sensitization to soy and wheat in the Multi-Centr. Allergy Study cohort. *Clin Exp Allergy* 2008: **38**: 493–500.
35. Liccardi G, Salzillo A, Piccolo A, Dàmato G. Skin prick test to horse should be included in the standard panel for diagnosis of respiratory allergy. *J Investig Allergol Clin Immunol* 2010: **20**: 93–4.
36. De Jong AB, Dikkeschei LD, Brand PLP. Sensitization pattern to food and inhalant allergens in childhood. A comparison of non-sensitized, monosensitized and polysensitized children. *Pediatr Allergy Immunol* 2011: **22**: 166–71.
37. Grönlund H, Saarne T, Gafvelin G, Van Hage M. The major cat allergen, Fel d 1, in diagnosis and therapy. *Int Arch Allergy Immunol* 2010: **151**: 265–74.
38. Mattsson L, Lundgren T, Olsson P, Sundberg M, Lidholm J. Molecular and immunological characterization of Can f 4: a dog dander allergen cross-reactive with a 23 kDa odorant-binding protein in cow dander. *Clin Exp Allergy* 2010: **40**: 1276–87.
39. Wiltshire S, O'malley S, Lambert J, et al. Detection of multiple allergen-specific IgEs on microarrays by immunoassay with rolling circle amplification. *Clin Chem* 2000: **46**: 1990–3.
40. Sanz ML, Blázquez AB, Garcia BE. Microarray of allergenic component-based diagnosis in food allergy. *Curr Opin Allergy Clin Immunol* 2011: **11**: 204–9.
41. Gaudin JC, Rabesona H, Choiset Y, et al. Assessment of the immunoglobulin E-mediated immune response to milk-specific proteins in allergic patients using microarrays. *Clin Exp Allergy* 2008: **38**: 686–93.
42. Ayuso R, Sánchez-Garcia S, Lin J, et al. Greater epitope recognition of shrimp allergens by children than by adults suggests that shrimp sensitization decreases with age. *J Allergy Clin Immunol* 2010: **125**: 1286–93.
43. Constantin C, Quirce S, Poorafshar M, et al. Micro-arrayed wheat seed and grass pollen allergens for component-resolved diagnosis. *Allergy* 2009: **64**: 1030–7.
44. Eckman J, Saini SS, Hamilton RG. Diagnostic evaluation of food-related allergic diseases. *Allergy Asthma Clin Immunol* 2009: **5**: 2.
45. Zijlstra WT, Flinterman AE, Soeters L, et al. Parental anxiety before and after food challenges in children with suspected peanut and hazelnut allergy. *Pediatr Allergy Immunol* 2010: **21**: 439–45.
46. Borres MP. Metachromatic cells and eosinophils in atopic children. A prospective study. *Pediatr Allergy Immunol* 1991: **2** (Suppl.): 6–24.
47. Irander K, Borres MP. An 18-year follow-up of allergy development related to nasal metachromatic cell findings during infancy. *Allergol Int* 2010: **59**: 193–200.