

REVIEW

Molecular diagnosis in allergy

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Clinical & Experimental Allergy

Summary

Development and progress made in the field of recombinant allergens have allowed for the development of a new concept in allergy diagnosis, molecular diagnosis (MD), which makes it possible to identify potential disease-eliciting molecules. Microarray-based testing performed with a small amount of serum sample enables clinicians to determine specific-IgE antibodies against multiple recombinants or purified natural allergen components. Performance characteristics of allergens so far tested are comparable with current diagnostic tests, but have to be confirmed in larger studies. The use of allergen components and the successful interpretation of test results in the clinic require some degree of knowledge about the basis of allergen components and their clinical implications. Allergen components can be classified by protein families based on their function and structure. This review provides a brief overview of basic information on allergen components, recombinants or purified, currently available or soon to become commercially available in ImmunoCAP or ISAC[®] systems, including names, protein family and function. Special consideration is given to primary or species-specific sensitization and possible cross-reactivity, because one of the most important clinical utility of MD is its ability to reveal whether the sensitization is genuine in nature (primary, species-specific) or if it is due to cross-reactivity to proteins with similar protein structures, which may help to evaluate the risk of reaction on exposure to different allergen sources. MD can be a support tool for choosing the right treatment for the right patient with the right timing. Such information will eventually give clinicians the possibility to individualize the actions taken, including an advice on targeted allergen exposure reduction, selection of suitable allergens for specific immunotherapy, or the need to perform food challenges. Nevertheless, all *in vitro* tests should be evaluated together with the clinical history, because allergen sensitization does not necessarily imply clinical responsiveness.

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Introduction

The discovery of IgE in the late 1960s introduced a new tool in allergy diagnosis, and determinations of specific IgE to different allergens were developed soon thereafter. In the early stages, radioimmunoassay was the preferred technique for measuring IgE, although it was later replaced by ELISA techniques. Recently, microarray technology has been introduced as a means of measuring specific IgE antibodies (Abs) against multiple recombinants or purified natural allergen components. This technique allows the testing of a large number of allergens with just a small amount of serum sample. Performance characteristics for allergens so far tested are comparable with the diagnostic tests currently used [1–4]. However, further studies testing more allergens are needed to con-

firm these results. Parallel to the development of new methods to measure specific IgE, there has been a revolution in the field of allergen extracts. In the beginning, crude natural and non-standardized extracts were used, although in time, the use of standardized extracts with a more precise content of allergenic components improved the diagnostic value of these determinations. The next quantum leap came with the use of purified proteins obtained from natural sources. Advances in the field of recombinant allergens led to the development of a new concept in allergy diagnosis, component-resolved diagnosis or molecular diagnosis (MD) in allergy [5, 6], allowing the identification of the potential disease-eliciting molecules. Recombinant allergens have also been used in a mixture with natural extracts (spiking) to increase test sensitivity.

The use of IgE epitope mapping of allergens using microarray-based immunoassay will probably be the next step in development [7]. In summary, in the last 40 years we have gone from allergen extracts containing a mixture of allergenic and non-allergenic moieties obtained by aqueous extraction of the source material to recombinant allergenic proteins or some of their peptides.

In the present review, I do not intend to review all the allergens described to date, but rather will focus on the clinical utility of allergenic molecules that are either currently available or soon to become available in commercial tests for the diagnosis of some allergic diseases.

Using allergen components in the clinic

In order to begin using allergen components and to properly interpret test results in the clinic, it is of help to learn some of the basics of allergen components and their clinical implications. First, it is important to know the allergen component names including their scientific acronym (e.g. Ole e 1 means allergen 1 from *Olea europea* or olive tree). Second, it is important to understand some properties of allergen components. Almost anything containing proteins can be an allergen source. Each allergen source contains many different allergenic proteins (allergen components). On each allergen component, there are commonly several different epitopes. An epitope is the actual three-dimensional binding site for the corresponding antibody. There is currently no known structure that is common to all allergen components or epitopes, i.e. there is no common feature that makes a substance an allergen or not. On the one hand, every species contain species-specific allergen epitopes, and antibodies formed to these structures bind only to the allergen epitopes in this particular species. On the other hand, proteins with similar structures are often present in biologically related species. Antibodies formed against such protein structures, can of course bind to the same or similar structure on a protein from several different species thereby causing cross-reactivity. Third, protein stability is another important aspect. Allergens that are stable to heat and digestion are more likely to cause a severe clinical reaction, whereas heat and digestion labile allergens are more likely to be tolerated or only cause milder/local symptoms. Consequently, it is important to know the protein structure and to which allergen protein family the component belongs as well as its stability to heat and digestion, because these features may affect the tolerance of different foods and the degree of severity of clinical reactions. Some food allergens may be tolerated raw, others need to be cooked. Some allergens will give rise to clinical reactions ranging from mild, moderate to severe, whereas others will cause sensitization without a clinical reaction.

There are several allergen databases that contain exhaustive information on allergens and protein families,

such as the official 'International Union of Immunological Societies allergen nomenclature database' – <http://www.allergen.org>, or the allergen literature database 'allergome' – <http://www.allergome.org> or the allergen database grouping the allergens into protein families, 'allfam' – <http://www.meduniwien.ac.at/allergens/allfam/>.

Species-specific allergen components are unique markers for its allergen source. The value of identifying species-specific allergen components is to single out the primary sensitizer that induces restricted reactions only to one specific source e.g. cat. Other allergen components are classified as markers for cross-reactivity due to their similar protein structures and IgE binding properties. They can be present in many different allergen sources, sometimes not even closely related. Identifying markers for cross-reactivity, on the contrary, gives valuable information on possible sensitizations to several different sources e.g. serum albumins from furred animals such as cat, dog and horse. One of the most important clinical utility of MD in allergy is its ability to reveal the allergens to which patients are sensitized, including primary or species-specific allergens and markers of cross-reactivity or pan-allergens. Identifying whether the sensitization is genuine in nature (primary, species-specific) or if it is due to cross-reactivity to proteins with similar protein structures may help to evaluate the risk of reaction on exposure to different allergen sources (Table 1).

Using microarray-based testing makes it possible to determine the IgE reactivity profile of a patient and assess their clinical sensitization pattern. The whole profile can give complementary information to the results achieved by single allergen components or extract-based testing. The choice of which allergen components to test should be based on clinical history, physical examination, previous test results and other factors such as age, geography and exposure among others. *In vitro* test results should always be evaluated together with the clinical history, because allergen sensitization does not necessarily imply a clinical reaction. The use of a predefined large number of allergen components in microarray systems can facilitate this task, although such an approach puts greater demands on interpretation.

Another area of research looks to establish whether information from MD can provide an indication as to the chances of tolerance development or if the allergy will be persistent (e.g. increased likelihood of persistent egg allergy if a patient is ovomucoid sensitized).

MD can also be a support tool for adapting treatment strategy to the particularities of each patient in a timely manner; doing so would eventually open the possibility of personalizing the actions to be taken. Some of these individualized actions would include targeted allergen exposure reduction advice, selection of suitable allergens for specific immunotherapy (SIT), or the need to perform food challenges.

Table 1. Important components by their ability to indicate primary sensitization or explain cross-reactivity

	Primary sensitization	Cross-reactivity
<i>Vegetable food</i>		
Peanut	Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 9,	Ara h 8
Soybean	Gly m 5, Gly m 6, Gly m 2S	Gly m 4
Wheat	Tri a aA_TI Tri a gliadin Tri a 19, omega-5 gliadin HMW-glutenin Tri a 14	
Buckwheat	Fag e 16kD	
Apple		Mal d 1
Peach	Pru p 3	Pru p 1 Pru p 4
Hazelnut	Cor a 8 Cor a 9	Cor a 1 Cor a 2 Cor a 11 Cor a 8
Kiwi fruit	Act d 1, Act d 2, Act d 5	Act d 8 Act d 1
Celery	Api g 1	Api g 1
Carrot	Dau c 1	Dau c 1 Dau c 4
Sesame	Ses i 1	Ses i 1
Brazil nut	Ber e 1	Ber e 1
Walnut	Jug r 1, Jug r 2, Jug r 3, Jug r 4	
<i>Pollens</i>		
Ragweed	Amb a 1	
Mugwort	Art v 1, Art v 3	Art v 3
Parietaria	Par j 2	Par j 2
Russian thistle or saltwort	Sal k 1	
Goosefoot or		
Lambs quarters	Che a 1	
Plantain or Ribwort	Pla l 1	
Timothy	Phl p 1 Phl p 5 Phl p 6	Phl p 4 Phl p 7 Phl p 11 Phl p 12
Bermuda grass	Cyn d 1	
Birch	Bet v 1 Bet v 6	Bet v 1 Bet v 2 Bet v 4
Alder	Aln g 1	Aln g 1
Oak	Que a 1	Que a 1
Olive	Ole e 1 Ole e 7 Ole e 9	Ole e 2 Ole e 7, Ole e 9
Japanese cedar, Cypress	Cry j 1Z Cup a 1	
Plane tree	Pla a 1 Pla a 2	
Latex	Hev b 1, Hev b 3, Hev b 5, Hev b 6	Hev b 5 Hev b 6 Hev b 8

Table 1. continued

	Primary sensitization	Cross-reactivity
<i>Animal Food</i>		
Egg white	Gal d 1, Gal d 2, Gal d 3, Gal d 4,	Gal d 5
Egg yolk	Gal d 5	
Milk	Bos d 4, 5, 6, 8, lactoferrin	Bos d 6
Shrimp	Pen a 1 Pen m 2 Lit v3 Lit v 4	Pen a 1
Cod and Carp	Gad c 1, Cyp c 1	Gad c 1 Cyp c 1
House dust mite	Der p 1, Der p 2 Der f 1, Der f 2	Der p 10
<i>Blomia tropicalis</i>	Blo t 5	
<i>Euroglyphus mannei</i>	Eur m 2	
<i>Lepidoglyphys destructor</i>	Lep d 2	
Cat	Fel d 1, Fel d 4	Fel d 2 Fel d 4
Dog	Can f 1, Can f 2, Can f 5	Can f 3 Can f 5
Horse	Equ c 1	Equ c 3
<i>Alternaria alternata</i>	Alt a 1, Alt a 6	Alt a 6
<i>Aspergillus fumigatus</i>	Asp f 1, Asp f 2, Asp f, 3 Asp f 4, Asp f 6	Asp f 6
<i>Anisakis simplex</i>	Ani s 1	Ani s 3
Cockroach	Bla g 1, 2, 4, 5	Bla g 7
Moth	Plo i 1	
Bee	Api m 1 Api m 4	CCDs
Wasp	Pol d 5, Ves v 1, 5	Ves v 2- CCDs

CCD, cross-reactive carbohydrate determinants.

Important allergen families

Allergen components can be classified as belonging to different protein families according to their function and structure, as shown in Table 2. In recent years, it has become clear that most allergens and their components belong to a limited number of protein families [8, 9]. As mentioned, IgE antibodies in the same protein family often show cross-reactivity, and classifying allergen components into protein families can aid in the answering questions on cross-reactivity.

Cross-reactivity between closely related molecular structures may shed light on several clinical syndromes such as oral allergy syndrome (OAS), latex–fruit syndrome and celery–spice–carrot–mugwort syndrome as well as the well-known cross-reactivity between Rosaceae fruits or between tree nuts. For example, profilins are proteins with a highly conserved structure and highly similar in their primary sequence not only present in virtually all pollens and plants contributing to cross-reactions between botanically unrelated species but also present in all eukaryotic cells.

Table 2. Allergen components classified as belonging to different protein families

Protein families	Characteristics	Allergens
Non-specific lipid transfer proteins	Stable to heat and digestion, causing reactions also to cooked foods. Often associated with systemic and more severe reactions in addition to oral allergy syndrome (OAS), and with allergic reactions to fruit and vegetables in southern Europe (not applicable to Par j 2 and Art v 3)	Ara h 9 Cor a 8 Pru p 3 Par j 2 Art v 3
Storage proteins	Found in seeds and serves as source material during the growth of a new plant. Often stable and heat-resistant proteins causing reactions also to cooked foods	<i>2S albumins</i> Ara h 2, 6 and 7 Ber e 1 <i>7S albumins</i> Ara h 1 Gly m 5 <i>11S albumins</i> Ara h 3 Gly m 6 Cor a 9 <i>Gliadins</i> Tri a 19
Pathogenesis-related protein family 10 proteins (PR-10)	Heat-labile proteins and cooked foods are therefore often tolerated. They are Bet v 1 homologues and often associated with local symptoms such as OAS and with allergic reactions to fruit and vegetables in northern Europe. May predispose to allergic reactions to Rosaceae fruits, hazelnut, carrot and celery	Bet v 1 Ara h 8 Gly m 4 Cor a 1 Pru p 1 Api g 1.01 Mal d 1 Act d 8 Dau c 1
Profilins	Actin-binding proteins showing great homology and cross-reactivity even between distant related species. Recognized as a minor allergen in plants and plant-related foods. Profilins are seldom associated with clinical symptoms, but may cause demonstrable or even severe reactions in a small minority of patients. Sensitization to profilin may give rise to multiple positivity when testing with plants and pollen extracts, however this has low clinical relevance in most cases	Bet v 2 Pru p 4 Hev b 8 Phl p 12
CCD	Can be used as a marker for sensitization to protein carbohydrate moieties (pollen, hymenoptera, etc.). Seldom associated with clinical symptoms, but may cause adverse reactions in a limited number of patients	CCD;MuxF3 Ana c 2

Table 2. continued

Protein families	Characteristics	Allergens
Calcium-binding proteins	Highly cross-reactive proteins present in most pollens, but not in plant foods	Bet v 4 Phl p 7
Serum albumins	Common proteins present in different biological fluids and solids e.g. cow's milk, beef, egg and chicken. Sensitization to serum albumins may give rise to airway reactions to mammalian animals, as well as food reactions to meat and milk	Fel d 2 Can f 3 Bos d 6 Sus PSA Equ c 3
Parvalbumins	Major allergens in fish. Also, a marker for cross-reactivity among different species of fish and amphibians. Parvalbumin proteins are stable to heat and digestion and therefore also cause reactions to cooked foods	Cyp c 1 Gad c 1
Tropomyosins	Actin-binding proteins in muscle fibres. Can be used as a marker for cross-reactivity between crustaceans, mites, cockroach and nematodes	Pen a 1 Der p 10 Ani s 3
Lipocalins	Stable proteins and important allergens in animals. Allergen components belonging to the lipocalin protein family display only limited cross-reactivity between species	Fel d 1 Fel d 4 Can f 1 Can f 2 Equ c 1 Mus m 1

CCD, cross-reactive carbohydrate determinants.

Cross-reactive carbohydrate determinants (CCD) are carbohydrate molecules attached to proteins present in all plants and in some insects and mites. IgE Abs to CCD may also help explain cross-reactivity.

Important allergen components

To date, many allergen components from different sources have been identified and characterized, and the list is rapidly growing in step with scientific progress. Interpretation of the information gathered from allergen component testing constitutes a huge step forward and the beginning of a new era in the field of allergy diagnosis. Important conclusions adding knowledge on the clinical utility of individual allergen components will be described in the following section. The most well-known allergen components are listed by plant origin and by animal/other origin in Table 1, and by protein family in Table 2. Both tables seek to give a brief overview of basic information including names, protein family and function, indication of likely primary sensitization and possible cross-reactivity. The tables contain components that are

currently either commercially available in the microarray format (ImmunoCAP, ISAC[®], Phadia, Uppsala, Sweden) or are most probably forthcoming.

Molecular diagnosis by allergens of plant origin

Foods of plant origin

Peanut (*Arachis hypogaea*) – Ara h 1, 2, 3, 6, 8 and 9. IgE ab to Ara h 1, Ara h 2, Ara h 3, Ara h 6 and especially Ara h 2, are regarded as markers for genuine sensitization to peanut [10–13]. They are stable proteins and therefore indicate an increased risk for systemic and more severe reactions to peanut [10, 14, 15]. Ara h 2 shows a high homology to Ara h 6 [11, 14]. Sensitization to several allergens is a stronger indication of more severe reactions than sensitization to only one of the components [10, 14]. Although clinically rare, sensitization to these peanut components may also give rise to a certain degree of cross-reactivity; for example, Ara h 1 to nuts and legumes such as lentil, pea and Gly m 5 from soybean [16, 17]; Ara h 2 to lupine and tree nuts such as almond and brazil nut [18]; and Ara h 3 to soybean, pea and tree nuts [19, 20]. Between 35–95%, 75–100% and 20–55% of peanut-allergic patients are sensitized to Ara h 1, 2 and 3, respectively. Ara h 1, 2 and 3 are generally stable to heat and digestion and therefore give rise also to a risk for reactions to cooked or roasted food.

Ara h 8 is a PR-10 protein, a Bet v 1 homologue, and thus a marker for primary sensitization through pollens like birch and alder. In a subgroup of patients allergic to birch pollen, the occurrence of peanut allergy might be mediated by means of cross-reactivity of Bet v 1 with the homologous peanut allergen Ara h 8 [21]. Cross-reactivity with lupine and Gly m 4 from soybean has also been documented [10, 22]. Ara h 8 is a heat-labile protein and cooked food is therefore often tolerated. In a population-based study, 95% of persons sensitized to Ara h 1, 2 and 3 reported symptoms after peanut intake. If only sensitized to Ara h 2 (not Ara h 1, 3 or 8), 87% reported symptoms, and in Ara h 8 monosensitized persons, only 18% reported symptoms [23]. In a recent study conducted in the UK positivity to Ara h 2 was the most important predictor of clinical allergy to peanut [24].

The presence of Ara h 9-specific IgE Abs is often associated with systemic and more severe reactions in addition to OAS [25], especially in southern Europe [26, 27]. Ara h 9 is an lipid transfer protein (LTP) protein and sensitization in most cases is probably due to primary sensitization to peach or other LTP-containing fruits.

Soybean (*Glycine max*) – Gly m 4, 5, 6 and S2. Gly m 4 belongs to the PR-10 protein family and is a major soy allergen in birch pollen-associated, soy-allergic patients. IgE Abs to Gly m 4 are likely due to primary sensitization

to birch or similar tree pollen and is often associated with mild symptoms [21, 28, 29]. Even though severe systemic reactions appear less likely, Gly m 4 has been shown to be a risk factor for severe OAS or systemic reactions to soy in birch-allergic patients [21, 30]. Gly m 4 is also cross-reactive with Ara h 8, and a relevant proportion – about two-thirds – of soy-allergic patients in Europe are associated with peanut and soy allergy [29, 31]. Targeted diagnostic testing with Gly m 4 is strongly recommended in pollen-sensitized patients with a suspicion of soy allergy, especially if the soy extract test result is negative. Some Gly m 4-sensitized patients can show low or even negative IgE results with soy-extract due to a low Gly m 4 content in the extract [31].

IgE Abs to Gly m 5 and Gly m 6 indicate primary sensitization to soy. Sensitization rates of 36% to Gly m 5 and 43% to Gly m 6 were recently reported in soy-allergic patients [29]. Anaphylaxis to soy was noted in 86% of those Gly m 5/Gly m 6-positive soy-allergic patients, whereas only 55% and 33% had moderate and mild symptoms, respectively. Hence, Gly m 5 and Gly m 6 are potential diagnostic markers for severe allergic reactions to soy [29].

Gly m 5 and Ara h 1 share sequence homology and so do Gly m 6 and Ara h 3. Serological cross-reactivity between these components has also been shown.

Wheat (*Triticum aestivum*) – Tri aA-TI, Tri a 18, Tri a gliadin, Tri a 19 (ω -5 gliadin) and high-molecular-weight-glutenin. A positive result to wheat-flour extract does not always correlate with clinical symptoms [32], indicating that *in vitro* diagnosis of allergy to wheat may be improved by using recombinant wheat-seed allergens [33]. The Tri aA-TI (α -amylase/trypsin inhibitor) protein fractions of raw and cooked wheat are a relevant wheat allergen in food allergy and also play a role in wheat-dependent exercise-induced anaphylaxis [34]. Positive IgE ab test results to Tri a gliadin indicate primary wheat sensitization with a low risk of pollen cross-reactivity [35]. The wheat component Tri a 18 (isolectine 1 agglutinin) and the latex component Hev b 6 are known to share sequence homology.

In children, IgE Abs to ω -5 gliadin (Tri a 19) are associated with a risk of immediate reactions to wheat [36, 37]. IgE Abs to Tri a 19 in adults are associated with a risk of exercise-induced reactions in connection with wheat ingestion, although the presence of the antibodies is not specific to this disorder in patients allergic to wheat [38, 39]. High-molecular-weight (HMW) glutenin is also a major wheat allergen and IgE Abs to HMW-glutenin are associated with wheat-dependent exercise-induced anaphylaxis [40].

Other important allergens, although not commercially available yet, are the 9 kDa wheat LTP, Tri a 14 in the albumin/globulin fraction and several low-molecular-weight glutenin subunits in the gluten fraction. All these

allergens showed heat resistance and lack of cross-reactivity to grass-pollen allergens. Wheat LTP is considered a major allergen only in patients living in southern Europe [34] and is also a significant allergen in baker's asthma in the same area [41, 42].

Buckwheat (*Fagopyrum esculentum*) – Fag e 16 kDa. – Buckwheat, a pseudo-cereal, has been recognized as a common food allergen in Asian countries. Fag e 16 kDa is a major allergenic protein of buckwheat. Fag e 16 kDa (2S albumin) has also been implicated as a major allergen in buckwheat allergy. Fag e 16 kDa shows similarities between BW 8 kDa from buckwheat, Ara h 6 from peanuts and Ric c 1 from castor bean [43] and appeared to be responsible for anaphylactic reactions [44].

Apple and Peach – Mal d 1, Pru p 1, 3 and 4. Because of cross-reactivity within the botanical family Rosaceae, the Mal d 1 and Pru p 1 components are good representatives and markers for some stone fruits such as cherry and apricot, among others, and thus not only for apple or peach [45].

Five allergy patterns were found in which the allergen families PR-10, LTP and profilin were involved. In the western Mediterranean area, allergies to Rosaceae fruits are caused by monosensitization to LTP (Pru p 3), monosensitization to profilin, or co-sensitization to both allergens [46]. On the contrary, monosensitization to PR-10, and to a lesser degree, co-sensitization to profilin and PR-10 is dominant in northern and central Europe. LTP sensitization is present both in pollinosis and non-pollinosis patients and is associated with peach allergy in particular. Patients sensitized to profilin are characterized by several concomitant allergies including grass and other pollens as well as Rosaceae and non-Rosaceae fruits. PR-10 sensitization is primarily associated with concomitant birch pollen and apple allergy [47].

IgE ab to Pru p 3, an LTP protein, is frequently associated with severe reactions not only to stone fruits but also to OAS [48], whereas sensitization to PR-10 proteins Mal d 1 or Pru p 1 and profilin (Pru p 4) is more often associated with OAS symptoms. LTP allergens of the Prunoideae subfamily have a similarity of about 95%, but there is also sequence homology of LTPs of botanically unrelated foods [49].

Hazelnut (*Corylus avellana*) – Cor a 1.0401, 8 and 9. A number of different IgE-binding components from hazelnut have been proposed as candidates for both sensitization and elicitation of allergic reactions. Some of the most intensively studied molecules in hazelnut over the last decade are the Bet v 1 homologue Cor a 1.04, the hazelnut profilin Cor a 2 and the LTP Cor a 8 [50]. Other molecules that have been investigated in connection with hazelnut allergy are the 11S globulin Cor a 9, the vicilin, Cor a 11, hazelnut oleosin, 2S albumins and the specific carbohydrate structures, known as CCD, for which bromelain has been used as the source. The presence of IgE to CCD did not

increase the ability to discriminate between patients with hazelnut allergy and hazelnut-tolerant patients. Sensitization to Cor a 1.04 is prevalent in the northern regions of Europe and is commonly associated to OAS. On the other hand, sensitization to hazelnut LTP (Cor a 8) is certainly more common in patients from southern Europe [51]. Patients sensitized to Cor a 8 may develop either severe or mild allergic reactions to hazelnut. In a recent study [51], no significant differences were found between different European regions regarding the prevalence of sensitization to Cor a 9 (11S globulin) among patients with hazelnut allergy and no differences were detected between patients with hazelnut allergy and control subjects with pollen allergy. It is still possible that Cor a 9 or other storage proteins are important to a relatively rare subgroup of patients with hazelnut allergy, although the condition appears to be prominent in the United States [52, 53]. Polysensitization to hazelnut allergen components is mostly observed in patients with severe symptoms [54].

Kiwi fruit (*Actinidia deliciosa*) – Act d 1, 2, 5 and 8. Kiwi allergy is not a homogeneous disorder because several clinical subgroups can be established. No definite allergen-recognition pattern was associated with the type of allergic reactions to kiwi [55]. It appears that when kiwi allergy was not associated with pollen allergy, these patients had the highest risk of systemic reactions to kiwi.

The two main kiwi fruit allergens are actinidin (Act d 1), a thiolprotease, and a thaumatin-like protein (Act d 2). The stability of Act d 1 and Act d 2 provides one explanation for the allergenic potency of kiwi fruit. Recently, a novel 40 kDa glycoprotein designated as Act d 3.02 has been described as an important allergen [56]. There are additional allergens described but its clinical relevance remains to be determined.

A potential association between respiratory allergy to cereal flour and allergy to kiwi fruit has been demonstrated. CCD and thiolproteases homologous to Act d 1 are responsible for wheat-kiwi cross-reactivity in some patients [57]. In patients with allergic reactions to figs and other tropical fruits (kiwi fruit, papaya, avocado, banana and pineapple), thiolproteases can mediate, at least in part, this cross-reactivity [58].

Kiwelin (Act d 5) is a novel protein of 28 kDa isolated from kiwi fruit (*Actinidia chinensis*). Serological tests and Western blotting analysis showed that kiwelin is specifically recognized by the IgE of patients allergic to kiwi fruit [59].

Bet v 1-homologous allergens (PR-10) from green (Act d 8) and gold (Act c 8) kiwi fruit are recognized by birch pollen/kiwi fruit-allergic patients in IgE immunoblots and ELISA experiments [60] and by ImmunoCAP using individual allergens [61]. These allergens may have relevance for patients sensitized to tree pollen and kiwi fruit, and might have been overlooked due to the underrepresentation of Act d 8 in the commercial extracts.

Celery (Apium graveolens) – Api g 1. Api g 1, the Bet v 1-PR-10, is the major celery allergen, although profilin (Api g 4) and CCD are also recognized by celery-allergic patients [62–65]. Api g 1 is more stable to heat than many other PR-10 proteins responsible for cross-reactivity with birch and mugwort pollen among other vegetables, although the structural similarity is less than for several other PR-10 proteins.

Carrot (Daucus carota) – Dau c 1. Dau c 1-PR-10 is the major carrot allergen and is responsible for cross-reactivity with Bet v 1 from birch pollen. However, in a subgroup of patients with carrot allergy, birch allergens did not inhibit IgE binding to Dau c 1 [66]. Profilin (rDau c 4) and CCD are also recognized by carrot-allergic patients [67]. Profilin may be an important allergen in allergy to carrot in birch-free areas.

Other seeds and nuts

Sesame (Sesamum indicum) – Ses i 1. The reactivity of the Ses i 1 (14 kDa, 2S albumin precursor) protein with most of the sera from patients allergic to sesame indicates that it is the major sesame allergen. However, other allergens have been isolated, such as a 7S vicilin-type globulin, a seed storage protein of sesame and named Ses i 3 and another 2S albumin, named Ses i 2 [68].

Seed storage proteins are known to be food allergens in peanut, walnut, Brazil nut and soybean. In particular, a strong correlation between IgE binding to these proteins and food-induced anaphylaxis has been demonstrated for Brazil nut and sesame seeds [69].

Brazil nut (Bertholletia excelsa) – Ber e 1. IgE to Ber e 1 (2S albumin) has been associated with clinical reactions to Brazil nut [70]. Ara h 2 shares common IgE-binding epitopes with almond and Brazil nut allergens. This may contribute to the high incidence of tree nut sensitization in peanut-allergic individuals [18].

Walnut (Juglans regia) – Jug r 1, 2, 3 and 4. IgE to the storage proteins of walnut is often associated with severe reactions [71]. Important walnut storage proteins that have been identified are Jug r 1 (2S albumin) [72], Jug r 2 (7S globulin) [73] and Jug r 4 (11S globulin) [74]. Walnut also contains the LTP Jug r 3 showing certain cross-reactivity with other LTPs, such as Pru p 3 [75]. Cross-reactions and/or co-sensitization between walnuts, other tree nuts and peanut are often observed [71]. Walnut and pecan nut belong to the same family, and cross-reactions often occur. Walnut also contains a PR-10-like allergen that may cause reactions to walnut in birch-sensitized patients [71].

Cashew nut (Anacardium occidentale) – Ana o 2. Ana o 2 is an 11S globulin storage protein and a major cashew nut

allergen [76, 77]. Structurally related cross-reactivity between storage proteins of the same protein family has been shown in 11S globulin legumin-like allergens in cashew nut (Ana o 2), peanut (Ara h 3) and other tree nuts (Jug r 4 of walnut, Cor a 9 of hazelnut) [20, 77].

Pollen

Weed pollen

Ragweed (Ambrosia artemisiifolia) – Amb a 1. Amb a 1 is a major allergen component in ragweed and about 95% of ragweed-allergic patients are sensitized to Amb a 1 [78]. It is considered to be a good marker for specific ragweed sensitization, thus indicating that SIT treatment with ragweed pollen allergens/extract is likely to be of clinical value.

Mugwort (Artemisia vulgaris) – Art v 1 and 3. Art v 1 (defensin) and Art v 3 (LTP) are major allergen components in mugwort [78–81]. IgE Abs to Art v 1 and/or Art v 3 can be used as specific marker allergens indicating that SIT treatment with mugwort allergens/extract is likely to be of clinical value if accompanied by compatible clinical symptoms with exposure.

About 80–95% of mugwort-allergic patients are sensitized to Art v 1 [82, 83]. Art v 3 is an LTP and a major allergen in the Mediterranean area with a reported sensitization prevalence of about 35–85% in Spanish mugwort-allergic patients [81, 84]. Art v 3 has been shown to display some limited cross-reactivity to *Rosaceae* fruit LTPs such as peach Pru p 3 and hazelnut Cor a 8, and may play a role in pollen–food syndromes associated with weed pollen [81, 85].

Parietaria (Parietaria judaica) – Par j 2. Par j 2 is a major allergen component in *P. judaica* (wall pellitory) showing great similarity to Par j 1 [86], and which can be used to identify primary *Parietaria* pollen sensitization suggesting the suitability of *Parietaria* SIT for the patient [87]. About 80% of Mediterranean *Parietaria* pollen-allergic patients are sensitized to Par j 2 [87, 88]. Par j 2 is an LTP, but cross-reactivity to fruit and vegetable LTPs is very limited [49, 89].

Russian thistle or saltwort (Salsola kali) – Sal k 1. Sal k 1 (peptid-methyl esterase) can be considered a specific marker for sensitization to *Salsola* pollen and other *Chenopodiaceae* (*Chenopodium album*) indicating suitability of the patient for saltwort SIT [90, 91]. This pollen is common in semi-desert areas.

English Plantain (Plantago lanceolata) – Pla l 1. Pla l 1 is the major allergen and is an Ole e 1-related protein from the olive tree pollen although this similarity appears to have little clinical relevance [92, 93].

Grass pollen

Timothy (Phleum pratensis) – Phl p 1, 2, 4, 5, 6, 7, 11 and 12. The degree of cross-reactivity between grass-pollen species is very high [94, 95]. However, some differences can be seen between subfamilies of grass.

Phl p 1 and Phl p 5 are major allergen components in timothy pollen [33, 95, 96]. They also have very high similarity with group 1 and 5 grass-pollen allergen components in other grass species, especially those belonging to the same subfamily (Pooidae) and Kentucky Blue grass e.g. Ryegrass. IgE Abs to Phl p 1 and/or Phl p 5 can be used as specific markers of sensitization to grass pollen, and suggestive clinical history may indicate that SIT treatment with grass-pollen allergens/extracts is likely to be of clinical value [97].

Phl p 4 (Berberine) is a marker of sensitization to non-pooideae grasses. However, Phl p 6 is contained in pooideae grasses, such as timothy or *Dactylis glomerata*, *Poa pratensis*, *Holcus lanatus* or *Lolium perenne*.

Patients sensitized only to Phl p 6 are likely sensitized to pooideae. Allergens from groups 1, 2, 5 and 6 are only expressed in grasses but not in other plants, so they detect a genuine sensitization to grasses. A patient sensitized only to Phl p1, but without IgE to Phl p 5 and Phl p 6, is likely sensitized to monocotyledon grasses such as *Cynodon dactylon* or *Zea mays*.

Phl p 7 (calcium-binding protein) and/or Phl p 12 (profilin) are markers for cross-reactivity, and if the patient has IgE Abs to these components but not to Phl p 1 and/or Phl p5, he/she is likely to be primarily sensitized to some other pollen [33, 95, 96]. Grass immunotherapy is then not recommended and the primary pollen sensitizer should be identified. Positive responses to calcium-binding allergens indicate possible cross-reactivity between pollen but not between plant food and pollen [98].

Phl p 11 is an allergen related to Ole e 1 and is similar to soybean tripsin inhibitor.

Bermuda grass (C. dactylon) – Cyn d 1. Cyn d 1 belongs to Group 1 grass pollens, the most frequently recognized grass-pollen allergens [94, 96]. It has partial cross-reactivity with Phl p1 [99], thus making Bermuda grass Cyn d 1 distinct from Timothy grass Phl p 1, and is suited as a marker of Bermuda grass pollen sensitization. Monosensitization to this pollen is common only in limited areas. Higher levels of IgE to Cyn d 1 than to Phl p 1 indicate primary sensitization to Bermuda grass, a pattern that is common in warmer climate areas.

Tree pollen

Birch (Betula verrucosa) – Bet v 1, 2 and 4. Bet v 1, a PR-10 protein, is the major allergen in birch pollen because about 95% of birch-pollen-sensitized patients show spe-

cific IgE to Bet v 1 [100, 101]. IgE Abs to Bet v 1 can be used as a specific marker for primary sensitization to birch pollen, especially in areas where birch trees are common, as in northern Europe [101, 102]. This fact indicates that SIT treatment with birch pollen allergens/extracts is likely to be of clinical value if respiratory symptoms coincide upon birch pollen exposure. It must be kept in mind that specific IgE to Bet v 1 can also be detected in patients with primary sensitization to other fagales. IgE Abs to Bet v 1 cross-react with PR-10 proteins present in other tree pollens (e.g. alder Aln g 1 and hazel pollen Cor a 1.01) and in many foods (e.g. hazelnut Cor a 1.04, apple Mal d 1, peach Pru p 1, soybean Gly m 4, peanut Ara h 8, kiwi Act d 8, celery Api g 1 and carrot Dau c 1) causing OAS in most cases [100–103].

IgE Abs to Bet v 2 (profilin) and/or Bet v 4 (calcium-binding protein) are markers for cross-reactivity [100]. If a patient has IgE Abs to these components but not to Bet v1, he/she is likely primarily sensitized to some other pollen. IgE Abs to Bet v 2 (profilin) are a marker of cross-reactivity between many pollens and plant foods [104], whereas IgE Abs to Bet v 4 are a marker of cross-reactivity between pollen allergens only [98].

Black alder (Alnus glutinosa) – Aln g 1; *Hazelnut tree (C. avellana)* – Cor a 1.01. Aln g 1 and Cor a 1 are PR-10 proteins with great similarity to Bet v 1 as well as Que a 1 (*Quercus alba*-oak). However, Que a 1 also shows some specificity for oak. Patients who react to allergens from other tree pollens of the Fagales order do so because of IgE cross-reaction with the major allergens of birch, alder, oak and/or hazel pollens [105].

Olive (Olea europaea) – Ole e 1, 2, 7 and 9. Ole e 1 [106] (trypsin inhibitor) is the most frequent sensitizing allergen from olive tree pollen and is regarded as a marker for primary sensitization to olive-tree pollen [100, 107]. Ole e 7 (LTP) and Ole e 9 (1.3- β -glucanase) are major olive allergens in geographical areas with high exposure to this pollen [105]. Exceptionally, these can be the only markers of sensitization to olive pollen [108]. Ole e 9 shares some common epitopes with β -glucanases from birch and ash pollens, tomato, potato, pepper, banana and latex [109]. Ole e 1 is also a marker for ash or Russian olive pollen sensitization [110, 111]. Sequence homology with English plantain Pla a 1 has also been shown [92]. Ole e 2 belongs to the profilin family and indicates cross-reactivity with other profilins [100].

Japanese cedar (Cryptomeria japonica) – Cry j 1, 2 and *Cypress (Cupressus arizonica)* – Cup a 1. IgE Abs to Cry j 1 are a marker for primary sensitization to Japanese cedar [112]. Sensitization to Cry j 1 in areas where Japanese cedar does not grow can be a marker for cypress sensitization instead, and a representative alternative to the

primary sensitizer in cypress, Cup a 1, because both are pectate lyases [113]. These cypress pectate lyases do not cross-react with the pectate lyase Art v 1 in mugwort.

Plane tree, Sycamore (Platanus acerifolia) – Pla a 1 and 2. The two major plane allergens are Pla a 1 (putative invertase) and Pla a 2 (polygalacturonase). Sensitivity to both Pla a 1 and Pla a 2 was 100% and 87.5% for mono-sensitized and polysensitized patients, respectively, with no false-positive reactions detected [114]. It appears that neither *P. acerifolia* profilin nor the two major allergens described (Pla a 1 and Pla a 2) can explain cross-reactivity with other plant-derived foods (hazelnut, banana, peanut and celery) [115]. However, the LTP, Pla a 3, may be involved in such food-pollen cross-reactions [116].

Latex (Hevea brasiliensis) – Hev b 1, 3, 5, 6 and 8. Detection of specific IgE to latex extract using traditional testing is frequently found in individuals without clinical symptoms to latex. Resolving the IgE sensitization into components is a tool to distinguish genuine latex allergy from sensitization to profilin. A profilin component (Hev b 8) is included in traditional extract-based tests; however, it is usually of low clinical relevance. On the other hand, sensitization to Hev b 1, Hev b 3, Hev b 5 and Hev b 6 is associated with primary latex allergy [117, 118].

Latex allergy occurs mostly among individuals exposed to latex in their occupation (e.g. health-care workers) or in children exposed to latex early in life, such as children who have undergone multiple operations, as in those with spina bifida. Latex allergy was a major health-care problem some decades ago, but increased knowledge and awareness have reduced both latex exposure and also the number of latex-allergic patients.

Hev b 1 (rubber elongation factor) is a major latex allergen. Sensitization to Hev b 1 has a high prevalence in children who have had multiple operations and spina bifida (50–100%) and prevalence is lower among health-care workers (10–50%) [119, 120].

Hev b 3 (small rubber particle protein) is a minor latex allergen. Hev b 3 and Hev b 1 are closely related and share stretches of sequence homology, a fact that may explain their cross-reactivity [121, 122].

Hev b 5 (acidic protein) is often associated with occupational latex allergy [111, 123, 124]. Hev b 5 has a significant homology with kiwi and potato, which are known to cause allergic reactions in latex-allergic patients [125].

Hev b 6 (hevein) is a major latex allergen with a prevalence of 70–90% among latex-allergic patients [126–128]. It is the main sensitizing allergen within health-care workers. Hev b 6 is also associated with the so-called latex-fruit syndrome (latex-avocado-kiwi-banana-chestnut) [128–130]. Hev b 6 shares sequence homology with Hev b 11, a chitinase, which may cross-react with chitinases in some exotic fruits.

Hev b 8 (profilin) is not associated with primary latex allergy, and it is a panallergen belonging to the profilin family [131]. Sensitization to profilin may explain serological cross-reactivity with other allergen sources of plant origin and is usually of low clinical relevance.

Cross-reactive carbohydrate determinants. Bromelin – MUXF3; Ana c 2. CCD is present in plant and insect glycoproteins (such as honeybees, wasps and cockroaches) carrying glycans with carbohydrate determinants that do not exist in mammals. CCD is rarely associated with clinical symptoms and can be used to resolve questions on non-symptomatic sensitizations obtained when testing with allergen extract-based IgE tests [132]. Approximately 20% of patients with multiple pollen allergies have IgE Abs to CCD [51]. A CCD test could be especially useful in four types of situations: (1) sensitization to foods of plant origin, (2) sensitization to latex in a pollen-allergic patient without occupational risk factors, (3) in subjects testing positive both for honeybee and for wasp venoms extracts and (4) in subjects with perennial respiratory symptoms who test positive for cockroach in the absence of demonstrable exposure to cockroach allergens. Ana c 2 (bromelin) and MUXF3 (processed from bromelain and usually coupled to a protein backbone for IgE testing) are both markers for sensitization to CCD. This fact indicates CCD-related cross-reactivity [133–135].

Also present in some plant proteins, O-glycans have IgE-binding capacity and clinical relevance, which differs from the aforementioned carbohydrates [136].

Recent studies have shown that IgE antibodies specific for the carbohydrate galactose- α -1, 3-galactose (α -gal) are capable of eliciting serious, even fatal reactions to the mAb cetuximab and to an unusual form of delayed anaphylaxis to meat (beef, pork or lamb). In contrast to previously described CCD motifs of xylose and core-3 – linked fucose, which are common in plants and insects, the α -gal epitope is abundantly expressed on cells and tissues of non-primate mammals. It appears that tick bites can give rise to IgE responses not only to tick-derived proteins but also to this type of carbohydrates [137].

Allergens of animal or other origin

Food of animal origin

Egg (Gallus domesticus) – Gal d 1, 2, 3, 4 and 5. Gal d 1 (ovomucoid), Gal d 2 (ovalbumin), Gal d 3 (ovotransferrin/conalbumin) and Gal d 4 (lysozyme) have been identified as the major allergens of egg white. Although ovomucoid comprises only 10% of the total egg white protein, it has been shown to be the dominant allergen [138, 139]. Gal d 5 is present in egg yolk as a livetin protein and in chicken as serum albumin [140].

Gal d 1, ovomucoid, has several unique characteristics, such as stability against heat and digestion by proteases, and is allergenic in minute amounts. IgE Abs to Gal d 1 is a risk factor for persistent egg allergy, and indicates that neither raw nor cooked egg is tolerated [141–144]. On the contrary, absence/low levels of IgE antibodies to Gal d 1 are associated with an increased probability of tolerance development [144], indicating tolerance to ingestion of cooked/heated egg [141, 142, 145]. Furthermore, quantification of Gal d 1 Abs is also useful in guiding the physician when deciding whether to perform a food challenge or not. Higher IgE ab levels of Gal d 1 indicate a higher risk of reacting to heated or raw egg, whereas lower concentrations indicate a lower risk of reaction to heated egg even if the patient might well react to raw egg [141].

Milk – Bos d 4, 5, 6, 8 and lactoferrin. Most milk-allergic patients are sensitized to several cow's milk proteins and there is no allergen component that can be considered solely responsible for cow's-milk allergy. A great variety is observed in the IgE Ab response.

The major allergens of milk are caseins (e.g. Bos d 8), β -lactoglobulins (e.g. Bos d 5) and α -lactoglobulins (e.g. Bos d 4), although allergies to other minor proteins such as bovine serum albumin (e.g. Bos d 6), bovine lactoferrin (Bos d lactoferrin) and Ig have also been reported [146, 147].

At 1.5–2 years of age, about 80% of children with milk allergy/intolerance gradually start to develop tolerance of milk, and milk allergy is less frequent in adults. Children outgrowing their milk allergy have IgE Abs primarily directed at conformational epitopes, whereas children with persistent milk allergy possess higher levels of IgE Abs directed to sequential epitopes [148–150]. Conformational epitopes are largely destroyed by high temperatures, and recently it was shown that a majority of milk-allergic children tolerated heated milk [151]. Those children reacting to heated milk had initially higher casein and β -lactoglobulin IgE levels and were at higher risk for systemic reactions. This study suggests two different phenotypes of milk-allergic children implicating different approaches for diagnosis and management.

Shrimp (*Penaeus aztecus*, *Penaeus indicus*, *Penaeus monodon* and *Pandalus eous*) – Pen a 1; Pen i 1; Pen m 1, 2; and Pen e 4. Several shrimp allergens have been identified to date [152]. Pen a 1 is a tropomyosin and a major allergen in shrimp [153–155]. It is detected in approximately 80% of shrimp-allergic patients and Pen a 1 from brown shrimp is regarded as a representative of other shrimp tropomyosins. Cross-reactivity between tropomyosins is very high and several studies implicate this phenomenon e.g. possible induction of shrimp allergy during mite immunotherapy [156] and occurrence of shrimp sensitization in an unexposed population of individuals due to tropomyosin [157].

Pen m 2 from black tiger shrimp is one of the recently identified arginine kinases indicating shellfish allergy and a potential representative for these new pan-allergens [158]. Lit v3 European white shrimp (myosin light chain with a high similarity to Bla g 8) and Lit v 4 (Sarcoplasmic calcium-binding protein) from *Litopenaeus vannamei* are the new allergenic shrimp proteins described [159, 160].

Fish parvalbumins from Cod (*Gadus morhua*) – Gad c 1 and Carp (*Cyprinus carpio*) – Cyp c 1. Cod Gad c 1 [161] and carp Cyp c 1 [162] are both major fish parvalbumin proteins and representative markers for fish sensitization in general [161, 163, 164]. Because of the high degree of cross-reactivity between parvalbumins from different species, 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 due to ingestion even after cooking, contact and inhalation of cooking vapour.

House dust mite – Der p (*Dermatophagoides pteronyssinus*) 1, 2 and 10; Der f (*Dermatophagoides farinae*) 1, 2, *Blomia tropicalis* Blo t 5. Mites belong to the most frequent and potent allergen sources world-wide. The most important house dust mite (HDM) is *D. pteronyssinus*, and in drier areas, *D. farinae*.

Der p 1 (cysteine protease) and Der p 2 (NPC2 family) are specific markers for sensitization to HDM [165]. More than 80–90% of HDM-allergic patients have IgE antibodies to one or both of these components [166, 167]. Therefore, Der p 1 and Der p 2 can be used as markers for specific sensitization and as an indication of usefulness of SIT to HDM [166]. High cross-reactivity among different pyroglyphidae dermatophagoid allergens from groups 1 and 2 have been demonstrated [167].

Der p 10 is a tropomyosin, but only about 10% of dust mite-allergic patients have IgE Abs to tropomyosin. A study suggested that exposure to HDM tropomyosin may sensitize against shrimp tropomyosin [157]. Potentially, patients with IgE to Der p 10 may have a higher risk of allergic reactions to shellfish (crustaceans and molluscs), insects and parasites [152].

B. tropicalis (non-Pyroglyphidae mite) Blo t 5 is a major allergen in patients sensitized to *B. tropicalis* [168]. It has partial cross-reactivity with allergens from Pyroglyphidae mites, and a positive reaction to Blo t 5 may suggest polysensitization to different mite species [169, 170].

Eur m 2 from *Euroglyphus mannei* (Pyroglyphidae mite) is a group 2 allergen with some cross-reactivity with other HDM. Lep d 2 from *Lepidoglyphus destructor* (storage mite) is a group 2 allergen with some limited cross-reactivity with HDM.

Furry animals

Cat (Felis domesticus) – *Fel d 1, 2 and 4*. *Fel d 1* (uteroglobin) is the major allergen component in cat, indicating primary sensitization. About 60–90% of patients with cat allergy have IgE Abs to *Fel d 1* [171–173]. IgE Abs to *Fel d 1* can be used as a specific marker indicating that SIT treatment with cat is likely to be of clinical value [171, 174, 175].

IgE Abs to the cat serum albumin *Fel d 2* are likely to cross-react with most other mammal albumins, such as dog *Can f 3*, horse *Ecu c 3*, pig *Sus s PSA* and cow *Bos d 6* [176, 177]. It can also cause reactions when eating pork (the cat-pork syndrome) [178]. About 15–40% of cat-allergic patients has IgE Abs to *Fel d 2* [176, 177].

Fel d 4 is a lipocalin protein shown recently to cross-react with major allergens from horse *Equ c 1*, dog or cow [179].

Dog (Canis familiaris) – *Can f 1, 2, 3 and 5*. *Can f 1*, *Can f 2* and *Can f 5* are specific allergen components indicating primary sensitization [180, 181]. *Can f 1* is a lipocalin protein and is the most relevant dog allergen and relatively stable to heat and in house dust [182]. *Can f 2* is also a lipocalin protein, and little is known about the cross-reactivity of these lipocalin dog-allergen components. However, *Can f 5* has been shown to cross-react with prostatic kallikrein in human seminal fluid [181]. About 50–90%, 20–33% and 70% of dog-allergic patients have IgE Abs to *Can f 1*, *2* and *5*, respectively [180, 181]. IgE Abs to *Can f 1* can be used as a specific marker indicating that SIT treatment with dog *Can f 1* is likely to be of clinical value [183]; however, more studies are needed to confirm this effect.

Can f 3 is the dog serum albumin protein, a cross-reactive component indicating cross-reactions to other bovine serum albumins e.g. from cat *Fel d 2* [176]. Most of the dog-allergic patients reacted to dog, cat and horse serum albumin [177]. About 50% of dog-allergic patients are sensitized to *Can f 3* [180]. If, for example, a patient is only sensitized to *Can f 3* and not to *Can f 1, 2* or *5*, the primary sensitizer needs ideally to be identified before giving advice on keeping or getting rid of the dog (also vice versa for cat, if only sensitized to *Fel d 2*).

Horse (Equus caballus) – *Equ c 1, Equ c 3*. *Equ c 1*, a lipocalin, is considered the major allergen of horse dander and has some cross-reactivity with mouse *Mus m 1* and cat *Fel d 4* [184]. *Equ c 3* is a serum albumin showing cross-reactivity with other mammals' serum albumins, as mentioned above.

Mouse (Mus musculus) – *Mus m 1*. Sensitization to mouse *Mus m 1* (calipocalin), as an indoor allergen, has been associated with asthma and asthma morbidity in some cities in the United States [185–187]. Occupational

allergy to mouse is fairly common in persons handling experimental animals.

Mould

A common and undesirable feature of mould allergies is the lack of a well-defined pathology and clear evidence of the disease. There are several reasons for this, such as heterogeneity of disease symptoms, differences in routes and amount of exposure and difficulties in manufacturing and standardizing fungal extracts. Non-standardized mould extracts may also result in poor outcomes in SIT [188]. Therefore, MD has the potential to substantially improve mould-allergy diagnosis and therapies [189–191].

Alternaria (Alternaria alternata) – *Alt a 1 and 6*. Sensitivity to *Alternaria* is a risk factor for development, persistence and exacerbation of asthma in both children and adults [192–194]. *Alt a 1* (acidic glycoprotein) is one of the most important mould allergens. It is a major allergen, which is recognized in approximately 80–100% of *Alternaria*-allergic patients [195–197]. *Alt a 6* belongs to the enolase protein family and may give rise to cross-reactivity among different species of mould.

Aspergillus (Aspergillus fumigatus) – *Asp f 1, 2, 3, 4 and 6*. Inhalation of the fungus *A. fumigatus* is responsible for many allergic respiratory diseases, the most notable being allergic bronchopulmonary aspergillosis (ABPA) due to its severity. *Asp f 1* (Robotoxin – mitogiline), *Asp f 2* (ASPND1), *Asp f 3* (perioxosomal protein) and especially *Asp f 4* and *Asp f 6* have been reported to be specific markers for ABPA [191, 198–200]. *Asp f 1* and *Asp f 2* are identified as major allergens in *A. fumigatus*. *Asp f 6* belongs to the manganese superoxide dismutase protein family and may give rise to cross-reactivity among different species of mould [190].

Cladosporium (Cladosporium herbarum) – *Cla h 8*. *C. herbarum* is a relevant allergenic fungal species that has been reported to cause allergic diseases in nearly all climatic zones. *Cla h 8* (mannitol dehydrogenase) is a major allergen, which is recognized by IgE antibodies of about 50% of all *Cladosporium*-allergic patients [201]. This is the highest percentage of patient reaction to any *Cladosporium* single allergen characterized thus far.

Insects and parasites

Anisakis (Anisakis simplex) – *Ani s 1 and 3*. *Anisakis* is a fish parasite that can cause severe reactions when eating raw infected fish [202, 203]. Larvae invading the gastrointestinal mucosa excrete/secrete proteins that are implicated in the pathogenesis of anisakiasis and can induce

IgE-mediated symptoms. Allergens Ani s 1 (serine protease inhibitor) and Ani s 4 have demonstrated their utility for diagnosing sensitization to the larvae of the genus *Anisakis*, but seropositivity for Ani s 1 has a limited diagnostic value in clinically discriminating patients with a history consistent with gastroallergic anisakiasis [204]. About 12% of the patients whose clinical history is compatible with gastroallergic anisakiasis recognize Ani s 5 as a unique allergen [205]. Ani s 2 [206] (paramyosin) and Ani s 3 (tropomyosin) [207] are also major allergens of *A. simplex* having extensive cross-reactivity with other tropomyosins from nematodes and invertebrates.

Cockroach (*Blattella germanica*) – Bla g 1, 2, 4, 5 and 7. Exposure to high levels of cockroach allergens in the home is a major risk factor for symptoms in sensitized individuals. The use of recombinant cockroach allergens may lead to the development of new approaches to asthma treatment in the future.

Previous results of skin tests and *in vitro* serologic assays have established that the prevalence of IgE antibodies to natural Bla g 1 and its homologue Per a 1 ranges from 30% to 50% among patients with positive skin-test responses to cockroach allergen. Previously identified allergens from *B. germanica* and *Periplaneta americana* include Bla g 2 (inactive aspartic proteinase), Bla g 4 (calycin), Bla g 5 (glutathione-S-transferase), Bla g 6 (troponin), the Per a 3 (arylphorin) and Per a 7 (tropomyosin) [208]. Serologic studies suggest that the prevalence of IgE antibodies to rBla g 4 and rBla g 5 is 60% and 70%, respectively, and that a cocktail of Bla g 1, Bla g 2, Bla g 4 and Bla g 5 would diagnose 95% of US cases of cockroach allergy [209].

Moth (*Plodia interpunctella*) – Plo i 1. The arginine kinase Plo i 1 is a marker for sensitization to moth and is also a likely marker for sensitization to other insects. Cross-reactivity with crustacea arginine kinases has been shown [210].

Hymenoptera venoms

Honeybee (*Apis mellifera*) – Api m 1, 4 and wasps (*Polistes dominulus*) – Pol d 5, (*Vespula vulgaris*) – Ves v 1, 5. Diagnosis of Hymenoptera venom allergy forms the basis for the immunotherapy treatment. Several major allergens, usually glycoproteins, have been identified in the venom of bees and vespids. The main species-specific major allergens are Api m 1 (phospholipase A₂) for honeybee, Pol d 5 for polistes, Ves v 1 (phospholipase A₁) and Ves v 5 (antigen 5) for vespulas [211]. Major allergens of different honeybees world-wide are very similar, and the structure of the major allergen phospholipase A₂ is highly identical. Antigen 5 is the major allergen in all vespid venoms [212]. There is also substantial cross-reactivity between *Vespula*, *Vespa* and *Dolichovespula* venoms.

Cross-reactivity of the Vespinae (*Vespula*, *Dolichovespula* and *Vespa*) with paper wasps (*Polistes*) is generally lower than cross-reactivity within the Vespinae. The cross-reactivity among European species of *Polistes* (*P. dominulus*, *P. gallicus*) is very strong, whereas that between European and American species is weaker [214]. Venom hyaluronidases (Ves v 2) have been identified as the most important allergens displaying CCD, whereas cross-reactions through the hyaluronidases' peptide backbones are less common. These allergens have low clinical relevance. Cases of double positivity of IgE to bee and *Vespula* venom, apart from true double sensitization, are often caused by cross-reactions, especially to CCD [213]. However, IgE to both recombinant Api m 1 and Ves v 5 indicates true double sensitization and immunotherapy is indicated with both venoms [214].

Api m 4, Melittin is a major bee-venom component, but only 28% of patients have specific IgE antibodies against this peptide [215].

Commentary and future work

The possibility of determining specific IgE antibodies against multiple recombinants or purified natural allergen components has allowed for the development of MD in allergy. This is also a new challenge for professionals involved in the care of allergic patients to properly interpret test results, clinicians must know about the basics of allergen components, their clinical implications and must always keep in mind that sensitization does not necessarily imply a clinical allergy. MD can be a support tool for reaching appropriate and timely clinical decisions on patients, which afford clinicians the possibility of individualizing the actions taken.

Although current microarray-based testing is able to detect one hundred different allergenic molecules, in the near future more molecules will be available for diagnosis. In all likelihood, IgE epitope mapping of allergens also using microarray-based immunoassay will be added.

Acknowledgements

We acknowledge Oliver Shaw for editorial assistance.

Disclosures: Joaquín Sastre reports having served as a consultant to Phadia, Schering-Plough and GSK, having been paid lecture fees by Novartis, GSK, Stallergenes, UCB and having received grant support from Phadia, GSK and ALK-Abello.

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