Food allergens - an overview on molecular properties and diagnosis

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Review article

Abstract

Food allergy is an increasing and potentially serious problem that can significantly limit patient's quality of life.

Classic allergy tests do not reflect the exact immunological reality of allergy. In severe cases the study of molecular components of food allergens allows to define the allergenic phenotype of each patient. It also allows a more accurate evaluation of cross-reactivity, improving patients follow up, with a more appropriate therapeutic decision of each case.

One of the most innovating tests in this area is ImmunoCAP® ISAC, which evaluates the patient's reactivity to several allergens in a single assay and using a small sample of serum.

Keywords: food allergy; molecular allergens; cross-reactivity; ImmunoCAP® ISAC

Introduction

Food allergy is an adverse reaction triggered when the immune system mistakenly recognizes a particular food or group of foods as aggressors to the organism[1].

The incidence of this disease has been increasing in recent decades. It is estimated that currently affects more than 17 million Europeans, including 3.5 million under the age of 25, according to the Food Allergy & Anaphylaxis Public Declaration (EACCI). The aetiology of food allergy is not completely defined, resulting from a complex interaction of genetic and environmental factors[2]. The food's fraction responsible for the allergic reaction is called allergen.

With advances in molecular identification of food allergens it is possible to relate the molecular structure of some of them with potentially aggressive structures of biological significance, namely molecules involved in defence against bacteria, fungi and insects, which present some form of thermal and proteolytic stability[3].

Definition

Food allergens belong to a restricted number of protein families that have a three-dimensional structure in common, and individuals with food allergies can manifest various types of immune responses to any of them.

The allergen sensitization through the gastrointestinal tract varies according to its molecular characteristics, which determine their stability to heat and enzymatic degradation, with the intensity of the allergic reaction being related to its lability[4]. Those that are easily destroyed by heat or digestion are less likely to cause significant adverse reactions when compared with more stable ones[5].

The major food allergens share in general a set of common characteristics, in that they are: soluble glycoproteins, size is between 10 and 70 kd, and relatively stable to heat, acidity

and proteases action[6]. Given these characteristics, various external constraints, such as food preparation, can affect the degree of allergenicity[6].

Understanding the concept of food allergen and the different allergenic component proteins within them is crucial to understand the phenomena of immunologic cross-reactivity, which is defined as the immunological recognition by an IgE antibody of similar allergenic molecules (homologues) present in different foods, either molecules of closely related species either molecules with similar functions present in different species[1] (figure 1). Major allergens with greater number of homologues responsible for cross-reactivity are summarized in table 1.

Ninety per cent of severe allergic reactions are caused by eight foods or food groups: cow's milk and its derivatives, egg, wheat, soy, fish, shellfish, peanuts and nut varieties[7]. The first four are associated with an earlier onset and usually transient allergy, while the remaining foods are associated to a later onset of the allergy, which tends to be persistent[7].

Classification

Food allergens are subdivided into two major groups, the animal origin and the plant origin. These large groups are composed of various subgroups of proteins that play different roles in the pathophysiology of food allergy.

The large families of proteins that constitute the allergen of animal origin are tropomyosin, parvalbumin and serum albumin. Tropomyosin is a binding protein in muscle fibres and plays a regulatory role in muscle contraction[6,7]. Its importance in this area comes primarily from the fact that it is an important marker of cross-reactivity between crustaceans, mites and cockroach. As it is stable to heat and digestion, reactions are common even with cooked food[6,7]. Shellfish allergy is more common in adults than in children, occurring more frequently in patients with concomitant dust mites allergy. Shellfish allergy is more frequent

than clams allergy, highlighting the shrimp as the most studied (allergenic component Pen a1). The great heat stability of tropomyosin makes it possible to trigger allergic reactions by inhalation[6,7].

The major fish allergen is parvalbumin and is a marker of significant cross-reactivity between different fish species and amphibians. It is also stable to heat and digestion, causing allergic reactions even with cooked foods. Fish is an important allergen source, especially in countries where its consumption is common. The first fish allergen purified and sequenced was cod's Gad c1, considered the reference model of sensitization to fish. Due to structural differences between the various parvalbumins, its importance as an allergen is not the same in all species of fish. Tuna and swordfish are the species with lower allergenicity due to a lower concentration of parvalbumin, making them tolerable for some patients[3,4].

Serum albumin represents a group of proteins present in different biological foods, such as milk, meat, eggs and chicken. The egg constitutes one of the main causes of allergic reactions in children. The egg white has a greater allergenic capacity than the yolk, and its main allergen element is the ovomucoid (Gal d1). The main characteristic of this element is its extreme resistance to heat and degradation by proteases, resulting in a great allergenic capability, even in small doses. On the other hand, the main allergen of the yolk is alpha-livetina (Gal d5), involved in the bird-egg syndrome. This syndrome involves the sensitization to bird's feathers by inhalation, developing later on allergic reactions to egg and poultry meat. There are also proven cross-reactivity phenomenon between the eggs of different types of birds. Other allergens present in the egg are ovalbumin (Gal d 2), ovotransferrin (Gal d 3) and lysozyme (Gal d 4). Cow's milk is very rich in proteins, particularly in casein (80%) and whey proteins (20%). The beta globulin is the whey protein that induces allergic reactions more frequently; however, 75% of patients are sensitive to more than one allergen component. The

phenomenon of cross-reactivity between cow's milk and goat's milk, sheep and horse are quite significant, with important clinical implications[3,4].

The proteins which constitute the allergens of plant origin are divided into three major groups: defence proteins, storage proteins and other large and heterogeneous group of structural, catalytic and regulatory proteins[8,9].

The group of the defence proteins (PR) includes components involved in the defence mechanisms of plants against pathogens or chemicals. Most of these proteins have a compact structure, stabilized by a significant number of disulfide bridges, making them highly resistant to chemical treatments and digestion by digestive proteases. All these features combined determine that they are immunologically active allergens, even in processed food, being able to induce a clinical response. The group of defence proteins are divided into 14 families, highlighting by importance and prevalence: PR-2, PR-3, PR-5, PR-10 and PR-14. The PR-2 (beta 1,3 glucanases) antifungal proteins are typically described as latex allergens. Recent data also suggested their presence in foods such as the banana, especially when it is very mature, as well as tomato, potato and pepper. The allergenic component present in all of them is Hev b2. Due to the fact of sharing the same allergenic component, these proteins are involved in the cross-reactivity phenomenon latex-pollen-food. The PR-3 (chitinase with hevein domain) is known to be mainly responsible for the latex-fruit syndrome. About 40 to 60% of latex allergy (allergenic component Hev b6.01) patients show hypersensitivity to vegetables, such as avocado (allergenic component Pers a1), chestnut (allergenic component Cas s5) and turnip (allergenic component bra R2). The PR-5 (thaumatins) represents antifungal proteins, and many induce a response to pathogenic attacks. These proteins are highly resistant to heat and proteases actions; they are present in kiwi (allergenic component Act c2), apple (allergenic component Mal d2), cherry (allergenic component Pru AV2) and pepper (allergenic component Cap a1). The PR-10 (Bet v1 homologous proteins) are a group

of thermo-labile proteins, therefore cooked food is usually tolerated. It's often associated with local symptoms, such as Oral Allergy Syndrome (OAS), as well as allergic reactions to pollens, fruits and vegetables. The cross-reactivity phenomenon most related to these proteins is the birch-to-rosacea ratio. Rosacea includes a vast group of plants prevalent in daily food, such as apple (allergen component Mal d 1), peanut (allergenic component Ara h8), carrot (allergenic component Dau c1) and soybeans (allergenic component Gly m4). The PR-14 (non-specific lipid transfer protein) constitutes a group of proteins stable to heat and digestion, causing reactions even in processed food. It's often associated with more severe and systemic reactions. These proteins are present in peach (allergen component Pru p3), apple (allergenic component Mal d3), orange (allergenic component Cit s3), hazelnut (allergenic component Cor a8) and corn (allergenic component Zea m14). They are found in greater amounts in the skin rather in the fruit's pulp. The comparison between the non-specific lipid transfer proteins (nsLTP) of the peach and the nsLTP of other plants demonstrates a very similar identity profile, making the Pru p3 an allergen representative of the nsLTP in food[3,4,8,9].

The storage proteins are an important group of proteins found in seeds that serve as a feeding source for a growing plant. The main groups of food that contain this type of proteins are cereals, dried fruits vegetables and spices. In general there are two super families: the prolamins and cupinas[8,9].

The prolamins are often stable and resistant to heat, and can cause reactions even in cooked food. The prolamins family includes some of the allergens responsible for allergic reactions caused by wheat (allergenic gliadin component w-5) either by wheat flour inhalation or by ingestion. Therefore food dependent exercise-induced anaphylaxis, frequently related to wheat, can be induced by its prolamins components. There is a great similarity between the y-

secalinas of rice and y3-hordein of barley, which explains the cross-reactivity between these two foods[8,9].

The cupinas undergo a series of structural adjustments when subjected to a significant rise in temperature, altering their immunological capacity. It was noted, for example, that roasted peanuts are more allergenic than those ingested raw, cooked or fried. These proteins are subdivided into three types; the leguminas are present in soybeans (allergenic component Glicilinas G1-G2), peanut (allergenic component Ara h3), brazilian nut (allergenic component Ber e2), hazelnut (allergenic component Cor a9), cashew (Ana allergenic component o2) and mustard (allergenic component Sin a2); the vicilin are present in soybean (allergenic component Gly m Bd), peanut (allergen component Ara h1), hazelnut (allergenic component Cor a11), cashew (allergenic component Ana o1), lentil (allergenic component Len c1), pea (allergenic component Pis s1) and sesame (allergenic component Ses i3); the germinas are present in orange (allergenic component Cit s1), wheat and pepper (allergenic component germ-line transcription (GLT)[3,4,8,9].

The profilins are proteins that act in the regulation of actin polymerization. They belong to the third group of heterogeneous structural, catalytic and regulatory proteins. They are rarely associated with clinical symptoms, but may cause moderate to severe allergic reactions in some cases. Their importance rests on the fact that they occur in pan-allergenic vegetables, found in pollens, food and latex. As such, the cross-reactivity phenomenon is frequent. As they are quite sensitive to heat and digestion, cooked food is generally well tolerated[3,4,8,9].

According to the information available in databases "Proteome" and "AllFam", referring to the family of allergens, is the prolamins super-family that contains the largest number of allergens, and profilins and lipid transfer proteins (LTPs) allergens that present a greater number of counterparts[10-12].

Molecular studies

Upon confirmation of allergy to a particular food, we should consider the investigation of other foods, including those potentially involved in triggering the allergic response. However, positive results to a specific food can simply represent a cross-reactivity reaction due to the presence of a homologous protein. Nuts, fish and shellfish are the foods that cause most often cross-reactivity phenomenon[7].

Certain proteins have identical amino acid sequences although not belonging to the same taxonomic classification. That is the case of pollen or latex with some fruits and vegetables. On occasion, the similarity between proteins occurs intra and inter-species, such as milk and beef meat and vegetables, respectively. This homology explains the importance of IgE in cross-reactivity: the sensitization to a particular protein can lead to allergic reactions when exposed to proteins with similar protein structures, not necessarily of the same allergen[13,14]. The prevalence and magnitude of allergic manifestations caused by proteins involved in the cross-reactivity phenomenon and by pan-allergens seems to be increasing and reflects an enhancement of atopy and allergic sensitization[14].

The limitations of the tests commonly used to establish the diagnosis and to characterize food allergies are greater when dealing with proteins involved in cross-reactions and with polysensitization cases[14]. The methods used for the diagnosis of food allergy are based on the observation of IgE-mediated allergic reaction or on the detection of specific IgE, related with a particular allergen used in the form of reactant, protein extracts or purified form. Assessments can be made *in vivo* (skin prick tests and oral provocation test) or *in vitro*. There are several *in vitro* assays available for specific IgE: Radio Allergo Sorbent Test (RAST), Enzyme immune assay (ELISA)[16], ImmunoCAP and Immuno Solid phase Allergen Chip (ImmunoCap-ISAC) (table 2). In RAST a radioactive marker is used to mark the IgE antibody bound to the allergen; the main disadvantage being the use of radioactivity. ELISA uses an

enzyme marker that can be measured by fluorescence; it has lower sensitivity than the more recent methods. ImmunoCAP uses a tridimensional cellulose structure that enhances the allergen-antibody bound and then an antibody enzyme marked with fluorescein[16]. ImmunoCAP-ISAC uses a miniaturized immunoassay platform of multiple allergenic components immobilized in a microarray supported by biochip automatized technology. Both ImmunoCAP and ISAC have high specificity and high sensitivity, although at low levels of specific IgE, ImmunoCap is more sensitive. The main advantage of ISAC is that only a very small amount of blood is needed to assess more than 100 molecular components; the main disadvantage is that it is a semi quantitative and quite expensive method[1,16].

The application of DNA technology (molecular biology and recombinant DNA techniques) allows the sequencing, synthesis and cloning of different proteins with allergenic capacity, with the resultant production of recombinant allergens. These recombinant molecules play a dominant role in the development of new tools capable of improving the diagnosis of allergy[15].

This new approach through the molecular study raises the diagnosis to a more specific level, allowing the quantification of specific IgE antibodies against molecules of pure allergens. Due to the possibility of producing individual allergenic components from a specific allergen source, the sensitization to these components is measured individually in separate tests, allowing to identify, at a molecular level, the component to which the patient is sensitive. These components also ensure additional information about risk, specificity and cross-reactity[16].

The latest methods consist in the use of preparations of purified allergens, allowing to perform their molecular study individually. One of the most innovative tests available on the market is in fact ImmunoCAP® Immuno Solid-phase Allergen Chip (ImmunoCap-ISAC). The ISAC system is routinely used in many allergy centres, allowing to examine whether

there is a sensitization to any of the more than 100 components derived from the 51 allergenic sources analysable by this technique, using only 20 to 30 uL of the serum or plasma of the patient, through a single assessment[10]. This test is a two-steps assay, where, initially, the IgE antibodies of the patient sample bind to the immobilized allergenic components and, subsequently, the IgE antibodies bound to the allergen are marked by a fluorescent anti-IgE antibody. Fluorescence is then measured using a laser scanner, and the results are evaluated automatically and customized by specific software, the Microarray Image Analysis (MIA). The results are given in standard units ISAC (ISU), indicating the levels of IgE antibodies within a measuring range of 0.3 to 100 ISU-E. The ISU-E is then normalized to the ImmunoCAP Specific IgE units. The test procedure takes a total time of less than four hours, and results from a combination of innovative biochip technology and pioneering research in the field of molecular allergology[16].

The advantages of using molecular allergens, and specifically ImmunoCAP® ISAC technology, are various; while the use of allergen extracts in the diagnosis of allergy only allows the holder to identify the source of allergen, recombinant molecules allows to create an allergenic profile of the patient. This is particularly important in cases of polysensitization, where it’s crucial to understand whether the response to a particular allergen is a phenomenon of co-sensitization or of cross-reactivity. It also allows the diagnosis using a small sample of blood from the patient, which is particularly important in the paediatric population. This test also enables a more accurate evaluation of the severity of allergy and further guiding the physician's intervention, either by introducing a prophylactic dietary restriction or by selecting specific immunotherapy[15].

Restrictions on specific food as therapeutic intervention in food allergies can lead to vitamin deficiencies, micro and macronutrients. In the particular case of the paediatric population, there is a significant risk of causing a negative impact on growth and

development; therefore children with food allergies under dietary restrictions should be followed and monitored on a regular and vigilant basis[17].

Molecular biology and genetic biotechnology using recombining allergens has also been used successfully in research, contributing to the detection, identification and molecular characterization of new allergens[10].

To establish a diagnosis based on molecular techniques a sound knowledge about the various groups of allergens, the particular clinical features and the elements most representative of each group are required. The molecular evaluation improves the quality of diagnosis when compared to the evaluation based on allergenic extracts. Furthermore, this approach allows to predict the possible intensity, and thus severity, of a clinical reaction[8]. The number of analysable allergens is increasing and the investment in perfecting these techniques has been growing. However, it's important to remember that the costs resulting from the use of molecular diagnosis tests are not negligible, and it's essential to establish a well-defined diagnostic plane, prioritizing the use of less expensive techniques in the simplest cases, which do not require such a thorough analysis of the allergenic profile.

This technique should be reserved for situations of complex allergies and polysensitization and always after conducting a thorough clinical investigation as first line approach and also after second line diagnostic exams like skin prick tests and other less expensive specific IgE tests [1,8,18,19].

Conclusions

The description of the allergenic profile of a specific food involves the identification of all potentially allergenic molecules contained in itself.

During the last decades the increasing use of molecular biology empowered the molecular characterization of numerous allergens, even those that are present in small quantities.

The proper approach of patients with complex food allergies is dependent on a complete diagnosis, providing a detailed list of specific molecular allergens, and their respective sources, that each patient should avoid. This is important not only for the averting of potentially hazardous food, but as well as to alleviate unnecessary dietary restrictions, which can negatively affect the quality of life. In addition, a clear diagnosis is also essential if specific immunotherapy is being considered.

Allergen testing technology is still evolving, with significant technical improvement and the possibility of increasing the number of analysable allergens, so that in the future the benefits can be even more meaningful and beneficial to patients.

There were no external sources of funding to implement this article.

The authors declare that they have no conflict of interest relating to this article.

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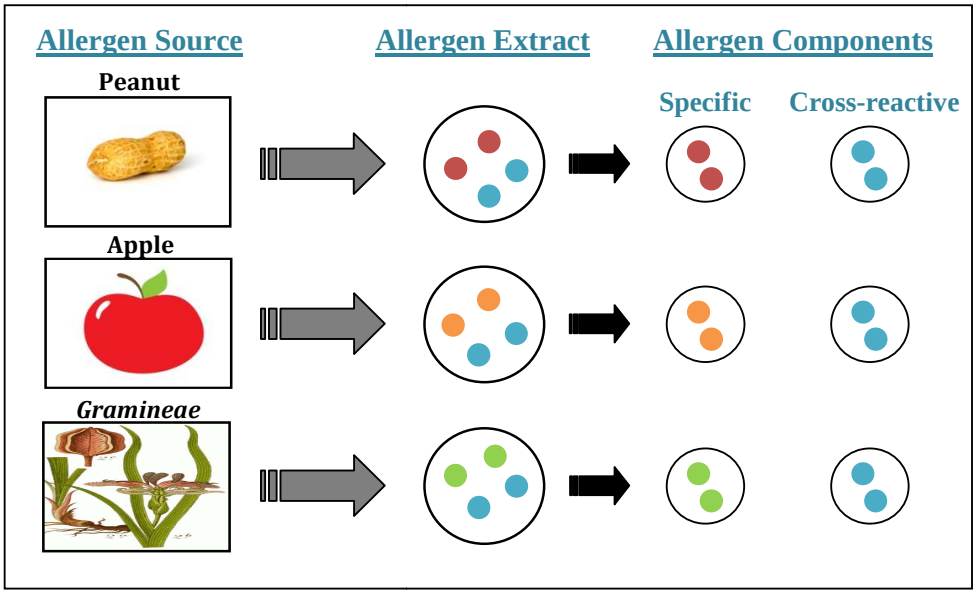

Fig. 1. The allergen source and its components

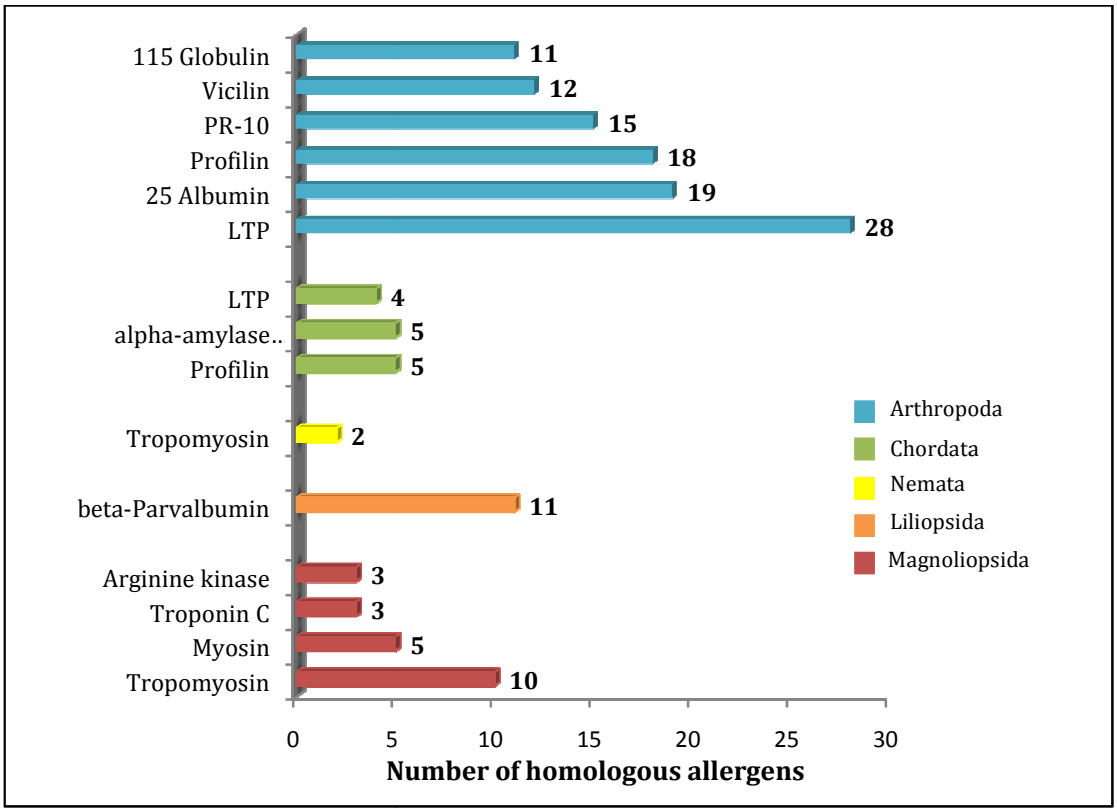

Table 1. Allergens with greater number of homologues responsible for cross-reactivity.

Type of *in vitro* test	Advantage	Disadvantage	Relevant Reference
RAST	Radioactive marker	Use of radioactivity	1; 16
ELISA	Enzyme immune marker	Less sensitive	1; 16
ImmunoCAP	Higher sensitivity and specifity	Quantitative	1; 16
ISAC	>100 components 30 µl of serum needed	Semi quantitative Very expensive method	1; 16

Table 2. Specific IgE *in vitro* assays