RECOMBINANT ALLERGENS IN DIAGNOSIS AND THERAPY OF ALLERGIC DISEASES

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ABSTRACT

The component-resolved diagnosis use in routine clinical and laboratory practice has increased in recent years. Recombinant allergens can be produced with high purity by using controlled procedures, obtaining molecules with known molecular, immunologic, and biological characteristics; they can help clinicians to treat patients with multiple pollen sensitisations. Recombinant allergens are useful in respiratory allergies such as: grass pollen, birch pollen, parietaria pollen, olive pollen, and dermatophagoides in food allergies, especially milk, eggs and peanuts. Recombinant allergens constitute an important tool in diagnosis and therapy of allergic diseases, which allows a better characterisation of the allergic patient.

Keywords: Recombinant allergens, allergy, diagnosis, immunotherapy.

BACKGROUND

In recent years, there has been an increasing use of recombinant allergens in routine clinical and laboratory practice, allowing a diagnostic approach of allergy at component level,[1] concurrently to the development of allergen microarrays. The ImmunoCAP® Solid-phase Allergen Chip (ImmunoCAP ISAC; Phadia, Upssala, Sweden) is the most common microarray that allows the detection of specific immunoglobin E (IgE) against a large variety of molecular components belonging to inhalant, food allergens and hymenoptera,[2,3] including: as species-specific components, as pan-allergens, or cross-reacting molecules, such as profilins, lipid transfer proteins, calcium binding proteins, storage proteins, tropomyosin, and serum albumins.[4,5]

Recombinant allergens can be produced with high purity by using controlled procedures that yield defined molecules with known molecular, immunologic, and biological characteristics.[6,7] Traditional allergen extracts, used for diagnosis and therapy, are prepared from natural allergen sources as a mixture of different species, which contain mixed allergenic components in undefined amounts of non-allergenic materials.[8-10]

Recombinant allergens are molecules that exactly mimic the properties of the natural allergens, or modified variants with advantageous properties, such as reduced allergenic activity or increased immunogenicity, or alternatively as hybrid molecules resembling the epitopes of several different allergens to include the relevant epitopes of complex allergen sources.[6,10]

The component-resolved diagnosis designates diagnostic tests based on pure allergen molecules, either produced by purification from natural allergen sources (designated according to the Allergen Nomenclature with the prefix 'n') or by recombinant expression of allergen-encoding cDNAs (designated with the prefix 'r').[11] These tests include marker allergens to diagnose the genuine sensitisation of patients towards a given allergen source or cross-reactive molecules that point to cross-sensitisation to several allergen sources.[12-14] This allows the accurate prescription of Sublingual Immunotherapy (SIT) for birch pollen,[12,13] grass pollen,[12] house dust mites,[15] and cats,[16,17] and includes

marker allergens for important Mediterranean pollen sources, including olive[18] and parietaria.[19,20]

Although allergenic source materials can contain just one major allergen (e.g. Bet v 1 from birch pollen), often several allergens are involved; 11 different allergens are characterised and cloned from sweet grasses, including Timothy-grass (*Phleum pratense*) and Ryegrass (*Lolium perenne*), while for the house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), the number is in excess of 20. Major allergens that account for the larger part of the IgE reactivity are at the basis of the development of therapeutic preparations.[21]

Recombinant allergens can help the clinicians in patients with multiple pollen sensitisations, who are frequently sensitised to several taxonomically unrelated allergens. Calcium-binding proteins and profilins are cross-reacting pollen pan-allergens: markers of multiple pollen sensitisation. Clinicians, if considering allergen-specific immunotherapy, have to establish whether sensitisation to several pollens is the result of co-sensitisation to different allergen proteins, co-recognition of homologous allergens, or both. So, detection of IgE reactivity to pan-allergens, and to major specific pollen allergens, is essential.[22]

GRASS POLLEN ALLERGY

Approximately 40% of allergic patients show IgE reactivity to grass pollens: one of the most important causes of IgE-mediated allergic disease in the world.[23,24] The most important source of grass pollen allergens in northern and central Europe is Timothy-grass (*Phleum pratense*).[25,26] Molecular and biochemical characterisation of *Phleum pratense*[25] has revealed several allergen components as rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5, rPhl p 6, rPhl p 7, rPhl p 11 and rPhl p 12: rPhl p 1 and rPhl p 5 are considered 'Species-Specific Allergens'.[27,28] The profilin rPhl p 1225 and the calcium-binding protein rPhl p 7 are the main cross-reactive components.[10]

The grass group 1 allergens are acidic glycoproteins (27–35-kDa), localised in the exine and cytoplasm of the pollen grain, with unknown function. Extensive immunologic cross-reactivity among the group 1 allergens from taxonomically-related grasses was established, and over 95% of allergic subjects were highly reactive to the respective group 1 allergens. The group 5 allergens include nine allergens with similar molecular mass to the group 1 (27–38 kDa); Phl p 5 has a ribonuclease activity, and its IgE-binding determinants are localised in the N-terminal and C-terminal ends. Approximately 80% of grass pollen allergic patients react with group 5 allergens, and together with the group 1, account for most of the IgE binding of most grass allergic sera: thus, they are considered major allergens.[12,27] Calcium-binding proteins such as Phl p 7 (8.6 kDa) are responsible for cross-reactivity between pollens of grasses, trees and weeds: antibodies against calcium-binding protein family were detected in 5-10% of pollen allergic individuals. Profilins as Phl p 12 (12-15 kDa) in the *Phleum* pollen are another group of allergenic molecules responsible for cross-reactions between various species of plants: Phl p 12 specific IgE antibodies account for cross-reactions with homologous profilins in many other allergens in both pollens and foods, and can be detected in 20–50% of grass-sensitive subjects.[27,29]

The predominant role of Phl p 1 and Phl p 5 in grass pollen allergy is demonstrated by many studies. Casquete-Román et al.[8] detected, in a paediatric population, IgE against rPhl p 1 and rPhl p 5 in 99.4% of the patients sensitised to grass pollen, while against rPhl p 7 and rPhl p 12, allergens were detected in 46% of them. Rossi et al.[27] found that in 77 adult patients (with a mean age of 21.6 years): rPhl p1 = 93.5%; rPhl p 5 = 72.7%; rPhl p 7 = 7.8%; and rPhl p 12 = 35.1%.[27] Successively, Rossi detected in 33 patients (with an age range of 9–62 years) rPhl p 1 in 100% of patients, rPhl p 5 in 76%, rPhl p 7 in 3%, and rPhl p 12 in 45%.[30] A study by Mari[28] found rPhl p 1 in 83%, rPhl p 5 in 50%, rPhl p 7 in 7%, rPhl p 12 in 15%, and isolated reactivity to rPhl p 1 in 6% in sera of 749 grass-sensitised patients (selected on a population of 4,606 unselected subjects, with an age range of 2-70 years).[10] Recently, Scaparrotta et al.[10] observed IgE against rPhl p 1 in 99% (205/207) of grass pollen-allergic children, to rPhl p 5 in 67% (139/207), to rPhl p 12 in 32% (66/207), and to rPhl p 7 only in 5% (10/207), with sensitisation only to 'Species-Specific' (rPhl p1, rPhl p5) allergenic molecules detected in 65% (135/207) of children. This study shows the predominant role of rPhl p 1 in paediatric populations as the most relevant sensitising allergen detectable at all ages and at all levels of Timothy-grass pollen-specific IgE antibodies, demonstrating that the importance of rPhl p 5 rises with the increase of patients' age and with grass pollen IgE levels.[10]

These observations confirm that the assessment of sensitisation to grass pollen allergenic molecules gives a better characterisation of allergic

sensitisation in grass pollen allergy in children, allowing a more specific and effective immunotherapy based on sensitisation to allergenic molecules.

The recombinant wild-type allergens and genetically modified hypoallergenic allergen derivatives can be used for immunotherapy, as for complex allergen sources with only one predominant allergen, if the relevant allergens have been included in the vaccine.[6] Although the first clinical immunotherapy study with recombinant allergen preparations used two different hypoallergenic derivatives of the major birch pollen allergen Bet v 1,[31] successively also *Phleum* recombinant allergens were successfully used.[32-35] Jutel and colleagues[32] demonstrated that a recombinant allergen vaccine with recombinant grass pollen, can be an effective and safe treatment to ameliorate symptoms of allergic rhinitis, associated with modification of specific immune response with IgG4 promotion and IgE reduction, consistent with the induction of IL-10–producing regulatory T cells.[32] Other authors confirmed these data, observing that patients with rhino conjunctivitis diagnosed using skin prick testing with a grass mix allergen extract and treated with a short course of SIT, based on a single species of *Phleum pratense* allergen extract, were able to develop an immune response that targets not only the immunising species, but also the grass mix allergen extract.[33]

BIRCH POLLEN ALLERGY

As in cold and temperate regions, birch pollen allergy affects approximately 20% of the population, e.g., in central and northern Europe,[36] the major birch pollen allergen, Bet v 1, is one of the first recombinant allergens extensively studied for allergy vaccination. Bet v 1 is the disease-eliciting allergen in approximately 90% of birch pollen sensitised patients and Bet v 1-cross-reacting allergens also cause symptoms to related tree pollen and foods.[37] IgE reactivity to the major birch pollen allergen Bet v 1 allows us to distinguish patients who are genuinely sensitised to birch pollen, while patients who exhibit positive skin tests to birch pollen extracts, but who have not been exposed to birch, have IgE to cross-reactive allergens, such as Bet v 2. So, the use of rBet v 1 is recommended in order to confirm the diagnosis of birch pollen allergy, before initiating immunotherapy.[38]

Bet v 1 vaccines, based on hypoallergenic recombinant rBet v 1, have demonstrated an improvement in allergic symptoms and favourably modify the immune response to Bet v 1. Vaccination with rBet v 1, formulated as tablets for sublingual administration, revealed clinically-relevant efficacy in rhino conjunctivitis patients, reducing symptoms and rescue medication compared to placebo.[37,39-43]

The profilin Bet v 2 is an actin-binding protein firstly identified as an allergen in birch, with homologous counterparts in a high number of pollens from phylogenetically-distant botanical families.[44] Detecting IgE reactivity to a single marker protein such as Bet v 2 is sufficient to diagnose or exclude sensitisation to profilins. Detecting IgE to multiple homologous, cross-reacting allergen proteins is not clinically more informative and increases the risk of confusion and misinterpretation.[45]

PARIETARIA POLLEN ALLERGY

Parietaria profilin Par j 2 might not share IgE-binding epitopes with profilins from other seasonal airborne allergens. Skin prick tests to *Parietaria* pollen is often negative in patients showing multiple pollen sensitisations, suggesting that Par J 2 might not always cross-react with profilins from other plant species. One study demonstrated that only less than 50% of patients hypersensitive to birch and grass profilins recognise this cross-reacting, ubiquitous allergenic protein in *Parietaria* pollen, and most of those who react to *Parietaria* profilin are sensitised also to the major, specific pellitory allergens, with practical relevance when the prescription of specific immunotherapy is considered.[46]

González-Rioja et al.[47] demonstrated that rPar j 2 displayed a 100% sensitivity and specificity among *Parietaria judaica*-allergic patients, supporting that *in vivo* and *in vitro* diagnosis could be simplified using rPar j 2, with comparable IgE response and skin prick reactivity of this protein with those produced by pollen extract.

A recent study demonstrated that a mutant hybrid, expressing genetically engineered forms of the major *Parietaria judaica* allergens (Par j 2/Par j 1), displayed reduced allergenicity and retained T cell reactivity for the induction of protective antibodies in vaccination approaches for the treatment of *Parietaria* pollinosis.[48]

OLEA POLLEN ALLERGY

Olive (*Olea europaea*) pollen allergy is one of the most significant pollinoses depending on geographical location. Although 10 allergens have been described from olive tree pollen, individual frequency of sensitisation can vary with the geographical area. Ole e 1 is the most prevalent allergen, observed in more than 70% among olive-sensitive patients, and the single major allergen in regions with low pollen counts, whereas other allergens such as Ole e 6, Ole e 7, and Ole e 9 also affect more than 50% of patients in locations with a high count.[49]

Ole e 1 is a single-polypeptide chain glycoprotein of 145 amino acid residues that constitutes more than 10% of the total protein content of pollen of the *Olea europaea* tree, but it does not exist in fruit, leaf, or stem. It has been demonstrated that the epitopes of Ole e 1 are only present in *Oleaceae* pollens[18] and subsequent studies demonstrated that sensitisation to Ole e 1 indicates primary sensitisation to *Oleaceae* pollens. Ole e 2 is a profilin and Ole e 3 is a calcium-binding protein with an amino acid sequence highly conserved in both taxonomically-related and non-related species: so, they are known as pan-allergens: markers of polysensitivity.[49]

Recombinant biotechnology offers most of the olive pollen allergens, with production of some hypoallergenic derivatives of Ole e 1: some of these molecules have been proven in a mouse model of allergy with promising results.[49,50]

HOUSE DUST MITE ALLERGY

rDer p 1 and rDer p 2 are the major recombinant allergens of house dust mite, and strongly correlate with *Dermatophagoides pteronyssinus* IgE. The lack of Der p 1 and Der p 2 IgE may help with differential diagnosis.[51] Both of these are proposed as promising hypoallergenic vaccine candidates for safer immunotherapy against house dust mite allergy.[52,53]

Der p 10 serum IgE prevalence and level suggest different patterns in food and mite-related tropomyosin sensitisation.[51] Der p 10 may be a diagnostic marker for patients with house dust mite allergy and additional sensitisation to other allergens. Such patients may require attention when allergen-specific immunotherapy is considered.[54]

FOOD ALLERGY

The use of recombinant allergens also represents a useful tool in food allergy. At first, its ability to reveal the exact allergen to which patients are sensitised (species-specific allergens or pan-allergens) is important in the evaluation of the potential danger of sensitisation and the risk of reaction on exposure. Sastre[55] focuses upon another area of research which looks to establish whether information can provide an indication as to the chances of tolerance development or if the allergy will be persistent.[55]

Milk contains more than 40 proteins. Casein (or nBos d 8) is a major allergen in milk and the main protein constituent of cheese.[56] It makes up about 75-80% of all milk proteins and is heat stable. nBos d 8 is subdivided into a number of families, αs1-, αs2-, β-, κ- and γ-caseins.[57] These are rapidly and extensively degraded by proteolytic enzyme during digestion. 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: it best discriminates between persistent and transient allergy,[58] it was often the cause of allergic reactions in patients with cow's milk allergy who eat so-called non-dairy products,[59] and in patients with a positive challenge to milk, nBos d 8 was the milk allergen component against which they most frequently had IgE.[60]

α-lactalbumin (or nBos d 4) represents about 25% of lactoserum (whey) proteins and approximately 5% of cow's milk proteins. It is the protein in highest concentration in human milk.[61] β-lactoglobulin (or nBos d 5) is the most abundant protein in whey, accounting for 50% of total protein in the lactoserum fraction and approximately 10% of cow's milk. The molecule nBos d 5 possesses 2 disulphide bridges and 1 free cysteine; this structure is responsible for the relative resistance of nBos d 5 to acid hydrolysis, as well as to proteases, which allows some of the protein to remain intact after digestion.[62] It has no homologous counterpart in human milk that does not contain β-lactoglobulin.[63]

Serum albumin (or nBos d 6), heat-labile protein, and lactoferrin are minor allergens.[64] The main allergens in egg are found in the egg white, but egg yolk also contains a large portion of specific IgE binding allergens. Gal d 1, Gal d 2, Gal d 3, and Gal d 4 are the most important allergens in egg white. Gal d 1 (ovomucoid) makes up approximately 10% of egg white and is often regarded the major allergen. Its allergenic potential is thought to

depend on its heat-stability and protease digestion. IgE binding activity to pepsin-digested ovomucoid have diagnostic value for distinguishing the challenge-positive subjects from the negative subjects: subjects with high IgE-binding activity to pepsin-treated ovomucoid are unlikely to outgrow egg white allergy.[64] Another study[65] concluded that patients with persistent egg allergy develop IgE antibodies against more-sequential and conformational epitopes of ovomucoid and ovalbumin, and that the presence of serum IgE antibodies to specific sequential epitopes of ovomucoid may be used as a screening instrument for persistent egg allergy.[66]

Gal d 2, also known as ovalbumin and albumin, is a major allergen of hen's egg white and is the most abundant of egg white proteins. A more recent study indicated that heated and ovomucoid-depleted egg white was less allergenic that heated egg white.[67] Gal d 3, also known as conalbumin, ovotransferrin, is a glycoprotein which is a present in egg white, egg yolk and plasma. Lysozimes (Gal d 4) are small globular proteins found in animal tissues and have concentration in egg albumen of about 0.5%. It is used as an additive and through this route may uncommonly induce symptoms of food allergy in sensitised individuals.

In children, positive IgE antibodies test results to Tri a gliadin indicate primary wheat sensitisation with a low risk of pollen cross-reactivity: in children, IgE antibodies to ω-5 gliadin (Tri a 19) are associated with a risk of immediate reactions to wheat.[68] In adults, they are associated with a risk of excercise-induced reactions in connection with wheat ingestion, although the presence of the antibodies is not specific to this disorder in patients allergic to wheat.[69]

Several proteins have been identified as peanut allergens, and the use of recombinant allergens has offered improved possibilities for a more specific and simplified peanut diagnosis.[70,71] Ara h 1, h 2, h 3 are seed storage proteins and have been designated as major allergens. Ara h 6 is a 2S albumin and shares several IgE epitopes with Ara h 2.[72,73] The minor peanut allergen Ara h 5 shows homology with pollen profilins and it is reported to be recognised by around 10% of peanut-sensitive individuals. Ara h 8, a Bet v 1-homologous pathogenesis-related (PR)-10 protein, has been shown as a major allergen for patients with concurrent birch pollen and peanut allergies. Lipid transfer protein (LTP), a pan-allergen with a degree of cross-reactivity comparable to profilin, is present in peanuts as Ara h 9. Measurement of IgE antibodies to rAra h 1, 2, and 3 is useful in the diagnosis of peanut allergy and in the investigation of reactions to raw and roasted peanuts. Some studies demonstrated that IgE antibodies to rAra h 2 are superior markers in their ability to differentiate between children in the allergic and tolerant groups, with a sensitivity and specificity of 88% and 84%, respectively (cut-off, 0.35 kUA/l). By combining the rAra h 2 and the rAra h 1 and rAra h 3 ImmunoCAP tests, it was possible to obtain an even higher specificity (94%).[74]

Peach is a well-documented and common cause of allergy in children, resulting in oral allergy and systemic reactions such as urticaria, asthma and anaphylactic shock, following the ingestion of fresh or processed fruit. Several peach allergens have been detected: Pru p 1 (PR-10 protein), Pru p 3 (a non-specific lipid transfer protein, Pru p 4 (a profilin) and Pru p glucanase. Pru p 3 is involved in allergenic relationship with other fruits from the family *Rosaceae*. A high level of cross-reactivity occurs among fruits and vegetables containing lipid transfer proteins, including sweet chestnut, cabbage, walnut, lettuce, and hazelnut.

CONCLUSIONS

In conclusion, there are strong data concerning the usefulness of recombinant allergens in diagnosis and the therapy of allergic diseases. These are two important tools that allow us to obtain a better characterisation of the allergic patient, resulting in a tailored treatment, specific for each patient.

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