

The Importance of Molecular Allergens for in vitro Testing: A Critical Evaluation of Component-resolved Diagnostics

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Introduction

Ten years have passed since the term Component-Resolved Diagnostics (CRD) was coined and the prospects of employing pure allergen components in IgE antibody based allergy diagnostics were comprehensively laid out [1].

CRD offers advantages over conventional, extract-based diagnostic methods in several ways, drawing from a few fundamental biological facts regarding the nature of allergens. First, purified recombinant or natural allergenic proteins present minimal biological and lot-to-lot variation as compared to natural allergen extracts. While variation in major allergen content of natural extracts may be monitored by extensive quality control procedures, the precise relative proportions of allergen components in an extract cannot easily be adjusted in most cases.

Secondly, while purified allergen components are typically stable and can be analysed by standard biochemical methods, proteins present in natural allergen extracts are exposed to different modifying and degrading enzymatic and biochemical activities from which they are compartmentally separated in the intact tissues of the allergen source. As a result, the allergen activity in a natural extract may rapidly decline unless protective measures are employed. Even if much progress has been made in achieving such protection, the measures available may not always be fully effective, potentially causing loss of certain sensitive components.

Thirdly, allergen components vary vastly in their properties in a variety of respects, including their abundance in the source material, their occurrence and structural conservation among closely and distantly related taxa and in different organs or tissues (e.g. pollen, fruits, pelt, urine), and biochemical stability. Sensitization to different allergen components will therefore have different implications with respect to origin of the sensitization and the probability of an allergic reaction on

contact with the allergen, as well as the likely nature of such a reaction, especially if the concentration of specific IgE antibodies is considered.

Diagnostic testing with pure allergen components therefore comprises reagents with a higher level of standardization and consistency than extract-based tests and offers the possibility of discriminating between different subtypes of allergic sensitization which may have direct implications for disease management. In addition, they may in certain instances provide for enhanced sensitivity and quantitative performance, as well as higher clinical specificity.

Diagnostic sensitivity enhancement

Enhancement of diagnostic sensitivity by the use of pure allergen components has been demonstrated several times in the recent past and new examples are underway. The improvement of a test for latex sensitization by complementation of natural latex extract with recombinant latex allergen *Hev b 5* (*rHev b 5*) was described in a previous volume of this series [2]. A similar improvement was reported for hazelnut [3], where the natural extract was complemented with *rCor a 1.04* in order to rectify a deficiency of this major allergen, causing a proportion of hazelnut allergic patients to pass undetected. Both these examples illustrate how specific deficiencies of natural allergen extracts can be addressed by the use of pure allergen components.

Superior diagnostic sensitivity of isolated allergen components in IgE antibody testing has been demonstrated in several food allergies, including cherry [4], hazelnut [5], apple and pear [6]. In these cases, *in vitro* IgE testing with recombinant major allergens enabled sensitization to be detected with a sensitivity equal to that of skin prick-prick testing with fresh food.

More efficient detection of allergen-specific sensitization through the use of more potent allergen reagents, such as pure allergen components in *in vitro* testing or fresh foods in skin testing, is almost inevitably associated with somewhat more frequent detection of subclinical sensitization in atopic individuals not displaying allergic symptoms to the allergen source under investigation. Even if the more important performance characteristic of a diagnostic test is its sensitivity (minimal occurrence of false negative test results), and although subclinical sensitization is a natural biological aspect of the biology of allergic disease and its underlying immunological mechanism, this is sometimes seen as a disadvantage of more sensitive diagnostic tests.

Subtypes of allergic sensitization and consequences for disease management

Diagnostic use of pure allergen components provides for the identification of different subtypes of allergic sensitization. Characteristics of such subtypes may be their cross-reactive potential or highly specific nature, their targeting of proteins that are highly abundant or scarce, or their origin as either a primary or a secondary sensitization. These different aspects of sensitization may be overlapping and dependent or discrete and independent of one another. In many cases, identification

of sensitization subtypes is of direct clinical relevance, for example in food allergy risk assessment or as guidance in avoidance, dietary or treatment strategies.

Both in food and respiratory allergy, sensitization to certain allergen components carries a different meaning and clinical consequence than sensitization to others. Several food allergen components have been found to be associated with a higher probability of significant, more severe or a specific type of food allergic reaction, as compared to other components in the same source or the entire food extract. For example, individuals sensitized to a non-specific lipid transfer protein (LTP) present in many fruits and nuts have been shown to frequently react with severe symptoms to these foods, including maize [7], lettuce [8], hazelnut [9–10], walnut [11], cherry [4], apple [12], grape [13], peach [14] and tomato [15].

Another example of severe food reaction associated with a particular molecular sensitization is wheat-dependent exercise-induced anaphylaxis (WDEIA). Most patients with this type of reaction to ingested wheat in combination with physical exercise show sensitization to the wheat allergen *Tri a 19* (ω -5 gliadin) [16].

In the more common problem of peanut allergy, sensitization to *Ara h 2* was found to be a particularly important determinant of clinical reactivity and additional sensitization to *Ara h 1* and *Ara h 3* enabled even stronger prediction of severe reaction to the same food [17].

A highly valuable differential diagnosis in peanut allergy is that between primary and secondary peanut sensitization in regions where birch trees are common. The former is characterized by IgE antibodies to *Ara h 1*, *Ara h 2* or *Ara h 3* and the latter by IgE antibodies to the PR-10 protein *Ara h 8*. While *Ara h 1*, *Ara h 2* and *Ara h 3* are highly abundant storage proteins, representing a high dose per unit of ingested peanut, *Ara h 8* is present in small amounts and targeted by a partially cross-reactive IgE response elicited by birch pollen allergen *Bet v 1*. As a result, individuals with a primary peanut sensitization are at greater risk of a significant reaction to peanut than those sensitized to *Ara h 8* alone and presumably to a smaller amount. While absolute avoidance at all times would be strongly recommended for peanut reactive individuals with a primary sensitization, a somewhat less rigorous instruction would still be considered safe for those with an exclusively birch pollen related, *Ara h 8* mediated peanut allergy as they are not likely to mount more than a local reaction on accidental contact with a low dose of peanut.

In pollen allergy, CRD may be of help towards identification of primary sensitizer(s) in regions of high botanical and aerobiological complexity and thereby provide guidance for immunotherapy treatment. Important groups of pollens, such as those of grasses, olive tree, plane tree, birch, mugwort, pellitory and saltwort, are characterized by a complement of both cross-reactive and more specific allergens. For example, while profilins and polcalcins are highly conserved and cross-reactive and occur ubiquitously in pollen across the entire plant kingdom, group 1 and group 5 grass pollen allergens are unique to grass pollen, *Art v 1* is unique to mugwort pollen, *Ole e 1* to olive pollen, *Sal k 1* to saltwort pollen and *Par j 1/2* to pellitory pollen. While sensitization to profilin and polcalcin cannot be traced to any particular pol-

len, the unique allergen components mentioned above may be utilized as markers for primary sensitization to their respective allergen source. As allergen-specific immunotherapy is intended to target major primary sensitization(s), selection of the most adequate treatment extract may be assisted by test results with such marker allergens in cases where there is otherwise uncertainty.

Concluding remarks

A growing body of evidence and experience adds to the increasing utility of component-resolved diagnostics in clinical allergy investigations. It is also evident that component-resolved diagnostics significantly contributes to our understanding of the complexity of allergy disease and the particular features of the underlying sensitization process.

Given that an increasingly comprehensive battery of allergen components will become readily accessible to a wider audience of clinicians and clinical researchers, there is every reason to believe that the coming decade will see a gradual shift from conventional diagnostic testing to component-resolved diagnostics. A driving force in this change of practice will be the development and acceptance of robust diagnostic concepts that can guide healthcare providers towards more personalized, adequate and cost-effective management of allergic patients and help improve patients' quality of life.

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Discussion

Fernandez-Caldas: Jonas, your comment about the hazelnut extracts can also go the other way round, meaning that it is much easier to make a hazelnut extract than a cherry or a pear extract because there is a lot more protein in these nuts.

Lidholm: Yes, but not all proteins.

Fernandez-Caldas: The detection for the hazelnut extract was close to 80% and the rest was 20%. So that definitely means that this hazelnut extract was much better in quality than the rest.

Lidholm: Yes, if you compare it like that you are right.

Fernandez-Caldas: So this point brings up the difficulties in preparing fruit extracts.

Lidholm: Yes, and that is actually a good remark because what we see in nuts, e.g., is the phenomenon of the balance of the protein composition if you like. There you

sometimes have cases where there is so much of one or two proteins that you mask out the other ones that you want to see there represented on your solid phase.

Carnes: What is the relationship between total extract, the whole extract and the purified protein? I do not know if you have a relationship because, e.g., of the whole extract you are using 5 mg while it is 1 mg of the purified protein? So, the differences cannot be explained. I do not know if you had measured the concentrations previously?

Lidholm: No, I cannot answer this question. I mean for the pure proteins of course, we can measure them exactly with common biotechnical techniques but the corresponding measurements on extracts would be very difficult and I just do not have that.

Carnes: But the concentration of the purified extract can be similar to the concentration in whole extract or not?

Radüge: I would like to ask a question concerning your statement that this higher specificity leads to a better choice of immunotherapy. I think as long as we do not have component resolved immunotherapy this is not true – or is there any evidence for that?

Lidholm: Yes, but you still have to pick the right extracts. You have to make a choice of which extracts you are using. So if you have an apparently polysensitized patient with specific IgE to five or so different pollens that can be caused by profilin reactivity or CCD or something else if you cannot provide evidence that it is actually one major allergen that sensitized that patient.

Radüge: Yes, but if the extract contains all allergens? I do not see any clinical evidence for this statement as long as we do not have component resolved immunotherapy.

Lidholm: Let us say the patient is sensitized to mugwort, to the major mugwort allergen plus profilin, e.g., and he is tested for grass and is positive there due to the profilin only. You could then be lead to the assumption that grass extracts are suitable immunotherapy reagents, while you in reality should be going for the mugwort extract.

Mari: Considering what we are discussing about the extracts, I mean standardization and so on, what I foresee is that if we go further with the CRD or microarray testing in the future, we are going to have to very carefully characterize a patient in terms of molecules. We are increasing the gap that is between our diagnosis and the therapy because the molecules for immunotherapy will come. But at the moment possibly what we need is to know the molecule in the extracts. That is my first comment. The second comment is about the sensitization, the immunology in the testing. I do not see this as a problem. Because in any...

Lidholm: But it is not a problem, it is a fact...

Mari: It is a fact, but we have to consider that any immune-based testing for autoimmunity, infectious diseases, etc. has this problem. That is not the problem, because you detect another condition that in the case of allergy could lead to mild symptoms, somehow, at high exposure and you do not have the history, if you did not ask for it. And then there is a phenomenon that is called facilitated antigen presentation. So if you have just one antibody binding your allergen this allergen is presented to the immune system easily, rather than others. So we have to consider that detecting IgE against one allergen is not all the problem if the symptoms are not there.

Pauli: It is just to give another response to my colleague from Germany. I think what we have to demonstrate when we inject pollen extracts which contain profilin – perhaps I can show it tomorrow – is that we can induce IgE against the profilin. But we do not know if there was any clinical significance. And this is a question and a remark for Jonas. I think what is also necessary in Europe that we know the marker allergen of ash and you then commercialize *Fra e 1*, but we have to say to the population and to the allergists that it is the same as *nOle e 1*. And often we have multi-sensitizations to many types of pollen, especially to birch and ash, which have the same season, and it is really important to have component resolved diagnosis for this. This is a concrete problem.

van Ree: So, Jonas, you know what you have to do when you get back...

