Recombinant allergen based approaches for the diagnosis of IgE-mediated type I allergies

by Dr. C. Harwanegg & Dr. R. Hiller

In the last decades single allergen molecules have been identified for many important sources of allergic diseases and the advent of recombinant DNA technology has since led to the characterisation of many of these allergens at the molecular level. Recombinant allergens can be arranged in diagnostic panels and used to resolve the IgE reactivities of an individual patient at the level of single disease-eliciting components, so called component-resolved diagnosis (CRD). This article reviews the advantages and shortcomings of CRD of allergy with purified or recombinant allergen molecules and discusses how this approach could change the way allergy is diagnosed and treated in the future.

IgE-mediated type I allergic diseases are amongst the most common causes of chronic illness in the populations of industrialised countries. Particularly in the last few decades, the prevalence of allergy has increased dramatically and accordingly the costs for diagnosis and treatment represent a major burden for the health care systems of the affected nations [1].

Once a patient has been sensitised upon the first encounter with an allergen, the disease is often aggravated following subsequent exposures and causes the well-known symptoms of allergy (e.g., skin rash, hay fever or chronic asthma). Hence, the identification of the origin of the allergic reaction is a key step in the diagnosis and treatment or to prevent further exposure.

The most prevalent sources of allergens are of biological origin, e.g., plant pollen, animal hair and dander, mites, moulds, or plant and animal derived food, and almost all of the relevant allergens are proteins [Figure 1]. More than a hundred years ago the first in vivo provocation tests were performed by applying crude preparations of tree pollen to the skin of an allergic patient. This simple test still represents the most popular procedure for the in vivo diagnosis of allergy (termed the skin prick test, SPT).

Since the discovery of immunoglobulin E (IgE) as the key mediator of the allergic response in the immune system [2], the routine determination of circulating IgE levels has improved the diagnosis of allergy significantly. In contrast to in vivo provocation, in vitro testing of allergen-specific IgEs reduces the risk of adverse reactions (e.g., anaphylactic shock) towards the allergen preparation being applied. In combination with a sound anamnesis, determining serum IgE levels is often sufficient to reliably identify the causative agent of allergy. At the present date, a large number of commercial suppliers are offering IgE assays and instruments and mostly they rely on the same basic test principle, the allergosorbent test [3]. A preparation of allergenic material is immobilised onto a solid phase carrier and incubated with serum from an allergic patient. The binding of allergen-specific IgEs is monitored subsequently by the addition of a specific anti-IgE antibody in combination with an appropriate signal generation and readout procedure. Along with the patient’s history and in vivo diagnosis, this testing procedure establishes the basis for the doctor’s decision towards an appropriate treatment.

This article reviews recent developments in diagnostic testing for IgE-mediated type I allergies. During the last decades, major advances have been made in allergen characterisation and standardisation, the adaptation of purified and recombinant allergens for diagnostic procedures and the development of novel test formats that facilitate the analysis of large numbers of allergens in a single step.

**Allergen standardisation**

Employing allergen extracts for diagnosis and treatment is widespread and supported by a long and successful clinical tradition. However, based on the knowledge that has been gathered during the last 20 years of in vitro allergy diagnostics, extract-based approaches for diagnosis and treatment are controversially disputed. Above all, biological extracts are heterogeneous mixtures of allergenic and non-allergenic components. The actual allergenic molecules may only account for a minor fraction of the total content of the extract. Most allergen sources (e.g., birch pollen, animal dander, storage mites, etc.) have at least several major and minor allergen molecules. Standardised mixtures containing all the relevant allergens in the appropriate (biologically and diagnostically relevant) concentrations are cumbersome or even impossible to produce and the performance of commercially available allergen extracts from different manufacturers can therefore vary significantly. This is due to several reasons. Firstly, some allergens might resist even sophisticated extraction procedures and may therefore be absent or under-represented in the resulting extract. The extractability of an individual allergen is a major prerequisite for test quality as it directly affects the assay sensitivity. Secondly, variable allergen stability leading to the partial or complete degradation of allergens during the extraction procedure could potentially interfere with the production of high quality extracts. Thirdly, biological materials are intrinsically heterogeneous regarding their allergen content. For example, when collecting tree pollen, the expression of certain allergens is affected by environmental factors, such macro- or micro-climate, pollution, or the developmental stage of the collected pollen. When considering food allergens, many different breeds exist for the common species and their allergen content is known to be different. In addition, biological raw materials may be contaminated by compounds of extrinsic origin. For example, animal hair and dander can contain mites and even a fraction of these alien allergens may eventually lead to false-positive results when the corresponding extracts are used for testing in certain allergic patients. Preparations of fruit or vegetable extracts can be contaminated with moulds or herbicides which can also induce false-positive results. Finally, many biological sources contain allergens with a strong potential for cross-reactivity. Cross-reactivity originates from IgE antibodies binding structurally similar epitopes from homologous proteins contained in different species. The use of heterogeneous extracts containing such structures bears the risk of producing false-positive or clinically irrelevant results.

During the last decade, a greater understanding of the problems mentioned above has led to numerous research activities focused on improving the stan-

---

**Figure 1. Overview of the most prevalent allergen sources in industrialised countries.**

As featured in CLI November 2004
Recombinant allergens

The advent of recombinant DNA technology in the early 1990s has led to a significant increase in the number of allergy eliciting molecules that have been characterised at the molecular level [reviewed in 5]. Before this time, the characterisation of allergen-containing biological materials was mainly based on Western blotting of serum reactivities. These investigations revealed that crude allergen extracts typically contain several allergens of major and minor relevance. In addition, the concept of allergen cross-reactivity had been established experimentally by immuno-blot inhibition experiments. The first reports of single allergen molecules produced recombinantly in E. coli appeared more than 10 years ago and since then the major disease eliciting components from the most important allergen sources (tree and grass pollen, food, animals, mites and moulds) have been characterised in molecular detail. Moreover, the elucidation of the biochemical function of many allergens as well as the characterisation of structural similarities has significantly improved the understanding of cross-reactivity, down to the level of individual epitopes [6].

Today, panels of recombinant allergens are available for many of the most important allergen sources. These panels are designed to assemble the epitope complexity of their biological counterparts. Testing with these purified recombinant (or natural) allergen components allows the majority of allergic patients to be diagnosed correctly and has several other benefits. First and most importantly, assay standardisation using well-characterised single molecules is superior to testing with even well standardised extracts and it also has positive effects on assay sensitivity and specificity. Moreover, the IgE-reactivities of an individual patient can be resolved at the level of individual disease eliciting components. Hence, the term Component-Resolved Diagnosis (CRD) was introduced for this particular diagnostic approach. In certain cases the resulting reactivity pattern of serum IgE can be used to improve the treatment for an individual patient significantly. For example, in the house dust mite (HDM) Dermatophagoides pteronyssinus more than 10 allergens have been identified so far [7].

Patients who are sensitised against HDM could be tested at least for the major allergens Der p 1 and Der p 2 as well as for Der p 10, a minor allergen with a high potential for cross-reactivity. Since HDM extracts are generally standardised only for their content of Der p 1 and Der p 2, a patient who does not have specific IgE against either of the two proteins will probably not benefit from specific immunotherapy with HDM extracts. On the other hand, if the patient sample reacts with both Der p 10 they are most probably sensitised against other mite species as well (e.g., D. farinae), and should not be treated with HDM extracts [7]. A similar constellation can be found in the European White Birch, Betula verrucosa. Extracts of this species produced for specific immunotherapy (SIT) are usually standardised against the major allergen Bet ν 1 to which more than 90% of all patients have specific IgE. Patients who are mono-sensitised against this particular allergen can therefore be considered highly suitable for receiving SIT. On the contrary, an exclusive sensitisation towards the minor birch allergen Bet ν 2 makes SIT not recommendable for two reasons. Firstly, the allergen might not be present to a sufficient extent in the extract used for therapy and secondly, Bet ν 2 belongs to the protein family of highly cross-reactive profilins that can be found in a wide range of biological sources. Therefore, cross-reactivity towards an allergen of a different biological source has to be considered as the primary cause of the reactivity against birch pollen extract.

In other cases, CRD may help to reveal the route of sensitisation of a particular allergen and this information can be used to prevent further allergen exposure more effectively. For example, in natural rubber latex (Hevea brasiliensis) reactivity against the major allergens Hev b 1 and Hev b 3 correlates with subcutaneous sensitisation that has been acquired via previous surgery. Patients with Spina bifida (SB) who typically have several surgical treatments in the first few years of life often react with these two allergens. On the other hand, the allergens Hev b 2, Hev b 4 and Hev b 6 are more frequently the cause of occupational allergy (e.g., in health care workers) who come into contact with latex products via skin exposure [8]. Moreover, a significant percentage of latex-extract positive patients may have been sensitised against a cross-reactive allergen, such as a profilin (e.g., Bet ν 2). Additionally, clinically non-relevant cross-reactions between so-called cross-reactive carbohydrate determinants (CCD) have been described as the cause of false-positive in vitro results when testing for latex.

Determination of the precise allergy eliciting molecule may even help patients to take preventive life-saving measures which is particularly important in patients who are prone to systemic reactions when they come into contact with a particular allergen. For example, allergens of the lipid transfer protein (LTP) family are frequently found in tree nuts (e.g., peanut and hazelnut) and fruits (e.g., peach and cherry) and these allergens are strong elicitors of life-threatening systemic reactions [9]. Other allergens of the same biological sources (e.g., Cor a 1, Ara h 1) have not been found to induce severe reactions in the affected patients. Therefore, a CRD with the corresponding allergenic molecules can be useful to identify patients who are prone of systemic reactions and to equip them with an appropriate emergency set.

Despite these promising results, CRD procedures are not yet established in routine diagnostic settings. Basically, this is for two reasons: firstly, even if a comprehensive IgE reactivity profile could be obtained with a panel of recombinant allergens, therapies based on the same molecules are still in a proof-of-concept stage of development. Secondly, only very few tests based on recombinant allergen panels are commercially available and these panels still lack important allergens. Allergy diagnosis on the basis of single molecules would require a large number of individual

<table>
<thead>
<tr>
<th>Assay type</th>
<th>Allergen binding</th>
<th>IgE binding</th>
<th>Signal amplification</th>
<th>Result generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beads</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microarray</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Comparison of three different technological solutions for IgE detection. Top - bead-based IgE detection: Microphers (beads) are coated with capture molecules, e.g., allergens, and incubated simultaneously with a liquid sample (e.g., human serum). A patient’s IgEs bind specifically to the allergen-coated beads and the interaction is monitored by the excitation of a fluorescence label contained in the secondary antibody. The individual beads are identified by a specific colour code while passing a secondary laser in a flow cyrometer. Up to a hundred reactions can be performed in a single analytical step.

Middle - microarray-based immunoassay: Capture molecules (e.g., allergens) are immobilised in an arrayed fashion on a functionalised glass slide and incubated simultaneously with patient serum. Allergen-specific IgE binding is monitored by the addition of a secondary anti-human IgE antibody containing a fluorescent label. Results are visualised by confocal laser microscope scanning and the biochip images are analysed with a customised software package.

Bottom - standard ELISA assay: Each well of a microplate is coated with an individual allergen and incubated - one at a time - with patient serum. Frequently, secondary antibody-enzyme-conjugates are used for detection that generate a photometrically quantifiable signal. Assays can be carried out in a high throughput fashion in microplates combined with automated read-out of well-specific signals.

As featured in CLI November 2004
allergens contained in the test format. For example, a comprehensive CRD-based test for allergy against grass pollen (e.g., timothy grass), mites (e.g., *Dermatophagoides pteronyssinus*) and latex (*Hevea brasiliensis*) would have to employ at least 30 individual protein components. Still this test would lack components of some other important species (e.g., other grass pollen and mites) that might be of relevance, and some naturally occurring allergens might contain allergens that have not been identified yet. As a consequence, a small percentage of patients would not be diagnosed correctly if not all the available allergens are employed in the assay. Moreover, for many natural allergens a plethora of isoforms has been identified and each of these isoforms might exhibit a different *in vitro* readout. It has also been shown that some recombinant allergens bind IgE with high affinity and resemble their natural counterpart quite well, whereas other artificial molecules only bind a minor fraction of IgE in contrast to the purified natural allergen. These differences mainly originate from certain post-translational modifications which are not an integral part of the gene expression machinery of the heterologous host where the allergens are expressed (e.g., *E. coli*) or from incorrect 3D folding of the molecular structure.

**Novel CRD-based allergy tests**

To develop CRD-based *in vitro* diagnostic tests presumably will require the implementation of novel test formats into routine diagnostic laboratories. Basically, assembling a comprehensive panel of recombinant allergens for diagnostic testing will require at least 50 and up to several hundred individual allergens, depending on the breadth of the diagnostic application. Currently, testing a large number of individual components poses several problems that must be addressed to successfully adopt CRD-based tests for routine allergy diagnosis. Firstly, state-of-the-art diagnostic instruments have been developed for the automated sequential processing of large numbers of patient samples but against single clinical parameters. In contrast to this, a CRD-based approach requires the simultaneous analysis of large numbers of allergens for a single sample. In addition, the latter has to be performed within an acceptable time frame, at a reasonable cost and using a minimal amount of patient serum. Typically, state-of-the-art instruments require about 50 μL of sample per individual allergen test. Using alternative formats that utilise assay miniaturisation (e.g., biochips, microbead assays) may permit the analysis of several hundred individual allergens with the same amount of patient serum [11] [Figure 2]. Secondly, although an increasing number of recombinant allergens are available for manufacturers, today’s list of components is far from comprehensive. Also, the recombinant production of a large number of allergens may be difficult as a growing number of molecules will require unique conditions for cloning and expression, quality controlled manufacturing and purification. Furthermore, many existing recombinant allergens are protected by patents and future manufacturers of comprehensive test panels may have to cope with a large number of different licensing agreements. Finally, CRD-based *in vitro* diagnostic tests may deliver very complex patterns of allergen-specific IgE reactivities. Therefore, medical professionals will have to be trained and assisted by expert software packages in order to interpret this information accurately and to choose - in combination with a thorough anamnesis and *in vivo* diagnosis - the most appropriate form of treatment.

**Conclusion**

Growing efforts in the molecular characterisation of allergens and the development of innovative assay designs will permit medical experts to use recombinant allergen-based approaches for the diagnosis and treatment of allergic diseases in the future. In combination with established standards, the interpretation of these novel test formats should be guided by diagnostic decision trees and guidelines that could be proposed by international committees of allergy experts. Allergy diagnosis has developed gradually over more than a hundred years and only during the last decades has *in vitro* IgE testing gained importance [Figure 3]. The introduction of recombinant DNA technology and the development of high throughput test formats in the 1990s have led to a significant acceleration and improvement in test standardisation and development. In order to fully exploit the potential of CRD-based IgE testing, the information that is generated by such tests must be correlated with *in vivo* results and the patient history. Presumably, large-scale studies will reveal reaction patterns that may help to improve the predictive value of allergy diagnosis by using component-specific diagnostic signatures for *in vitro* testing, disease prevention in early life and monitoring of specific immunotherapies. Combined efforts in allergy research and clinical diagnosis will lead to a better understanding of the effectiveness of single allergen molecule-based approaches and will help to improve the treatment of allergic diseases significantly.

**References**


**The authors**

Christian Harwanegg, Ph.D. and
Reinhard Hiller, Ph.D.
VBC Genomics Bioscience Research LLC,
Rennweg 95 B,
1030 Vienna, Austria
Tel.: +43 1 796 65 72 52 (CH)
Tel.: +43 1 796 65 72 51 (RH)
Email: christian.harwanegg@vbc-genomics.com
Email: reinhard.hiller@vbc-genomics.com

---

Figure 3. Simplified overview of the historical development of allergy diagnosis and treatment.