Recombinant allergens provide new opportunities

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Today allergenic proteins can be identified and produced in large quantities by recombinant DNA technology. Recombinant allergens can be produced with consistent quality and without genetic and biological variation. Using recombinantly produced allergenic proteins in *in vitro* testing provides new opportunities for designing improved tests and offers new tools to address clinical and immunological questions.

Recombinant allergens provide new opportunities to refine the diagnostic procedures of IgE mediated allergy. In addition to pointing out the offending allergen source, e.g. birch pollen, it is now possible to also identify the actual protein components eliciting the allergic symptoms. Thus, *in vitro* tests based on recombinant allergens are useful tools to collect information on symptom triggers at the molecular level. Recombinant *in vitro* tests make it possible to study more complicated phenomena, such as geographic differences in clinical reactivity and cross-reactions towards seemingly distant allergens.

**Birch allergen components**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Common name</th>
<th>Biological function</th>
<th>Mw (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bet v 1</td>
<td>PR-10 protein</td>
<td>Pathogen response protein</td>
<td>17</td>
</tr>
<tr>
<td>Bet v 2</td>
<td>Profilin</td>
<td>Actin-binding protein</td>
<td>15</td>
</tr>
<tr>
<td>Bet v 3</td>
<td>4-EF-hand calcium-binding protein</td>
<td>Calcium-binding protein</td>
<td>23</td>
</tr>
<tr>
<td>Bet v 4</td>
<td>2-EF-hand calcium-binding protein, polcalcin</td>
<td>Calcium-binding protein</td>
<td>8</td>
</tr>
<tr>
<td>Bet v 6</td>
<td>Isoflavone reductase</td>
<td>Isoflavone reductases</td>
<td>34</td>
</tr>
<tr>
<td>Bet v 7</td>
<td>Cyclophilin</td>
<td>Petidylprolyl isomerase</td>
<td>18</td>
</tr>
</tbody>
</table>

Traditional allergy tests merely point out the offending allergen source, e.g. birch pollen. But such an allergen source in fact contains a number of allergenic components. With tests based on recombinant allergens the specific proteins responsible for the patient’s sensitization can be identified.
**Improved allergy tests**

Recombinant allergens can be used to design improved allergy *in vitro* tests:

**Improving the clinical sensitivity of natural extracts**
In cases when the natural extract has a scarcity of a specific allergenic component, addition of this recombinant protein to the extract improves the clinical sensitivity and the quantitative performance of the test. Phadia has already used this strategy with great success to design an improved version of ImmunoCAP™ Allergen k82, Latex, ensuring that all sensitized patients are really captured.

**Designing optimized recombinant “extracts”**
Recombinant allergens may be combined to form a well-characterized composition containing an optimal amount of relevant allergenic components of a natural extract, but excluding components of little or no diagnostic value. This offers interesting opportunities for future test development.

**The use of recombinant allergens in IgE antibody tests**

![Illustration of recombinant allergens in IgE antibody tests]

*As an additive to extracts*  
*As extract replacement*  
*As single allergen components*
New tools to answer clinical questions

– single component tests

By using tests for single allergenic components as a complement to more traditional IgE antibody tests, further clinically relevant information can be gained. The possibility to investigate the sensitization to single allergenic components can shed light on phenomena that have hitherto been difficult to explain. Some examples:

Explaining geographic differences in clinical reactions

In the northern part of Europe the characteristic symptoms of allergy to fruit and vegetables are local reactions in the mouth or throat (oral allergy syndrome, OAS), while patients in southern Europe more frequently have systemic symptoms. The explanation appears to be different sensitization profiles. Testing with recombinant single component tests shows that Bet v 1-sensitization, in all probability caused by birch pollen, dominates in the north. In the southern parts, on the other hand, antibodies to lipid transfer proteins (LTPs)* dominate, indicating the predominance of true food allergy.

Explaining clinical reactivity

to enable better advice to patients

Testing with single components is a useful tool to investigate and explain allergic reactions more in detail and to determine if they are caused by cross-reacting IgE antibodies to different allergens.

For a patient showing symptoms when eating apples or other fruits, traditional extract-based tests will determine the source of the allergen triggering the reaction, such as apple, pear or cherry. However, the original source of sensitization could also be tree or grass pollen and the symptoms due to a cross-reaction between allergenic components with similar structures present in both plant pollen and food proteins.

Tests for single allergenic components can be used to give additional information on the source of sensitization on the molecular level – e.g. pollen molecules, LTPs or profilins – and make it possible for the physician to draw conclusions as to the clinical implications. Whereas pollen sensitized patients with symptoms during specific seasons may benefit from symptomatic treatment during the pollen season, avoidance of the offending foods may be essential for patients with LTP sensitization. These patients are also likely to develop more severe symptoms.

* LTPs are major allergen components in many fruits, such as peach, apple, apricots, and widely distributed throughout the plant kingdom.

LTPs from botanically unrelated vegetables show a moderate to high degree of sequence homology.
Optimized patient selection for immunotherapy

Recombinant allergens enable a more specific diagnosis which greatly improves the diagnostic base for prescribing specific immunotherapy (SIT):

Determining the sensitization profile before treatment

Single recombinant allergen tests offer new opportunities to determine whether a patient is a good candidate for SIT or not, and for suggesting the optimal therapy.

If the patient’s allergic reactions are caused by sensitization to the major allergen components of a common allergen source (e.g. Bet v 1 in birch pollen), the patient is likely to respond well to immunotherapy with common extracts, as these contain a high amount of this component.

On the other hand, if the patient is sensitized to another component than the major one, e.g. Bet v 2 or Bet v 4 in birch pollen, immunotherapy with extracts heavy on Bet v 1 will probably not be effective enough. There are even concerns that immunotherapy with allergenic components to which the patient is not sensitized may in fact induce new sensitization that may worsen the symptoms rather than reducing them.

Monitoring treatment

When SIT is used, the immunological effect of the treatment can be followed periodically by determining IgE and IgG antibodies to major recombinant allergens. Furthermore, periodic determinations also make it possible to spot potential development of sensitization to minor cross-reacting components.

A possible decision tree for the treatment of birch pollen allergy

Confirm birch pollen sensitization
ImmuNoCAP™ Allergen t3, Birch

+  

↓

IgE-mediated birch pollen allergy confirmed

Clinically relevant birch pollen allergy unlikely

Component resolved diagnostics:
- Major birch pollen component: ImmunoCAP™ Allergen t215, rBet v 1
- Cross-reactive minor birch pollen components: ImmunoCAP™ Allergen t216, rBet v 2 and t220, rBet v 4

Suitability for a birch pollen specific immunotherapy?

<table>
<thead>
<tr>
<th>Allergen: Result:</th>
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</tr>
</thead>
<tbody>
<tr>
<td>rBet v 1 +</td>
<td>rBet v 1 +</td>
<td>rBet v 1 −</td>
</tr>
<tr>
<td>rBet v 2, 4 −</td>
<td>rBet v 2, 4 +</td>
<td>rBet v 2, 4 +/−</td>
</tr>
</tbody>
</table>

High | Medium | Low
**CRD – the new approach in allergy diagnostics**

Recombinant allergens make component resolved diagnostics, CRD, possible. With CRD the total antibody reactivity profile of an allergic patient may be identified, along with the disease-eliciting allergens and potential cross-reactivity interactions. CRD has the potential to revolutionize allergy diagnostics. In the future, diagnoses based on detailed individual reactivity profiles at molecular level, may enable specific immunotherapy with the exact proteins that the individual has become sensitized to, and much better and more precise advice on allergen avoidance.

**Phadia leads the way**

Phadia has long been at the frontline in the field of recombinant allergens, offering IgE antibody tests with recombinant and purified natural allergen components. Over 10 years ago, the first recombinant allergen on ImmunoCAP™ assay platform was introduced. Since then, the number of single component diagnostic *in vitro* tests is continuously growing.

Our scientists are busy developing new recombinant allergen tests, investigating their clinical importance and exploring ways to utilize them. We are convinced that recombinant allergens will become important tools not only for research purposes and specialized applications, but also in clinical routine IgE antibody testing, for the benefit of wider circles of physicians and patients.
**What is a recombinant allergen?**

A recombinant allergen is a biotechnologically produced allergen molecule originally identified from an allergen extract. Most of the existing recombinant allergens have been expressed in *Escherichia coli* and are usually comparable with their natural templates (proteins) in structural features and immunological properties. Other high-technological expression systems have been developed to produce recombinant allergens through bacteria, yeast and insect cells. Recombinant allergens mostly have immunoglobulin E (IgE) antibody binding capacity comparable to that of the natural allergen and generally show good reactivity in *in vitro* and *in vivo* diagnostic tests.

To date, many different allergen components from various allergen sources have been cloned, sequenced and expressed as recombinant proteins.

**Literature:**