Occupational Allergens: Is there an Application for Recombinant Allergens?

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Many occupational agents, particularly HMW proteins (like wheat/rye flour components, enzymes, laboratory animal urinary proteins, wood proteins, mite components, natural rubber latex etc.) and some LMW, cause OA by sensitization with the production of specific IgE antibodies.
Relevance of single/recombinant allergens for the diagnosis of Baker’s asthma

• is one of the most common form of occupational asthma affecting up to 15% of bakers, millers and pastry factory workers

• causative allergens: proteins from wheat and rye flour and some baking additives such as $\alpha$-amylase
Wheat seeds are composed of different protein classes:

- the water-/salt-soluble *albumins* and *globulins*
- the ethanol-soluble *gliadins*
- the urea, detergent or KOH soluble *glutenins*

The most relevant allergenic wheat fractions for baker’s asthma are the water-/salt-soluble *albumins* and *globulins*.

2-D gel electrophoresis wheat flour water-/salt-soluble fraction
Identified wheat single allergens:

- $\alpha$-amylase inhibitors (MW 14-17 kDa)
  - Tetramer WTAI: CM2, CM3B, CM16, CM16*
  - Homo-dimer WDAI: WDAI-1, WDAI-2
  - Monomer WMAl

- Peroxidase (MW 36 kDa)

- Agglutinin homo-dimer (MW 17 kDa)

- LMM glutenin, $\alpha$-gliadin, $\gamma$-gliadin, $\omega$-gliadin (especially for food allergic)

- Glycerin-aldehyd-3-phosphat-dehydrogenase
  - Triose-phosphat-isomerase,
  - Serpin
  - Acyl-CoA-oxidase,
  - Fructose-bisphosphat-aldolase
Summary for baker's asthma

- All soluble fractions contain allergens; the most relevant for baker's asthma are the **albumins** and **globulins**.

- In 2D-immunoblot of the salt-/water-soluble fraction more than **100 different allergen spots** were detected.

- The **allergen spectrum** is individually **very different**.

- So far, no common major wheat allergen could be identified.

- The use of **recombinant allergens** for diagnosis of baker's asthma seems to be **not the first choice**.

- Nevertheless, it is necessary to **optimize the quality** of the test extracts.
Relevance of single/recombinant allergens for the diagnosis of latex allergy !?
**Hevea brasiliensis latex allergens**

<table>
<thead>
<tr>
<th>IUIS name</th>
<th>description</th>
<th>MW (kDa)</th>
<th>incidence in latex</th>
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</thead>
<tbody>
<tr>
<td>Hev b 1</td>
<td>Rubber elongation factor (REF)</td>
<td>14.6</td>
<td>Rubber particle</td>
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<tr>
<td>Hev b 2</td>
<td>β-1,3-Glucanase</td>
<td>34-36</td>
<td>B-Serum</td>
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<tr>
<td>Hev b 3</td>
<td>Small rubber particle protein (SRPP) (prenyl transferase)</td>
<td>24-27</td>
<td>Rubber particle</td>
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<td>Hev b 4</td>
<td>Micro helix</td>
<td>50-57</td>
<td>B-sera</td>
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<td>Hev b 5</td>
<td>Acid protein</td>
<td>16-24</td>
<td>C-sera</td>
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<tr>
<td>Hev b 6.01</td>
<td>Prohevein</td>
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<td>B-sera</td>
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<tr>
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<td>Hevein</td>
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<td>C-domain of the prohevein</td>
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<tr>
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<td>Hev b 8</td>
<td>Profilin</td>
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<td>Enolase</td>
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<td>Hev b 12</td>
<td>Lipid transfer protein</td>
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<td>?</td>
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<tr>
<td>Hev b 13</td>
<td>Esterase (ENSP)</td>
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<td>B-sera</td>
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</table>
Prevalence of specific IgE antibody to individual Hev b allergens in latex allergic health care workers (HCW) (n=104)*

- rHev b 1: 11.5%
- nHev b 2: 73%
- rHev b 3: 7.8%
- rHev b 5: 67%
- rHev b 6.01: 67%
- rHev b 7.02: 26.1%
- rHev b 8: 12.5%
- rHev b 9: 1.8%
- rHev b 10: 2.8%
- rHev b 11: 12.5%
- rHev b 12: 10.2%
- nHev b 13: 83.2%
- HRP/CCD: 11.5%
- MBP: 0%

*HCW: German n=51; Portuguese n=21; American n=32; Raulf-Heimsoth M et al. Clin Exp Allergy 2007; 37: 1657-1667
Comparison of the ImmunoCAP-results of k82 without rHev b 5 („old“) and of k82 with rHev b 5 („new“) (68 sera of latex allergic HCW)
Summary for latex allergy

✓ Sera of latex allergic patients clearly recognize major latex allergens.

✓ Single recombinant allergens are useful to study sensitization profiles.

✓ A mixture of four recombinant allergens improved the in vitro NRL-sIgE-determination compared to the k82, but it was less efficient than the NRL-preparation supplemented with rHev b 5.

✓ A new approach to improve in vitro-allergy diagnostics: if a relevant allergen component is present only in suboptimal amount in a natural allergen extract, that component can be added as a stable recombinant protein to the extract preparation during production.
Summary

• with exception of natural rubber latex there is **no immediate application** of **recombinant allergens** in the diagnostic of occupational allergy,

• but nevertheless it is **absolutely necessary** to improve the **in vivo** and **in vitro tools** for diagnosis of occupational allergy.