Entomophagy: what about allergies?

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What about entomophagy?
ENTOMOPHAGY IS GLOBAL

80 percent of nations eat insects in one way or another. That’s more than 2 billion people worldwide!

Source: Food & Agriculture Organization of the United Nations
http://www.provivam.com/
### Why ?

**ADVANTAGES** | **DISADVANTAGES**
---|---
Nutritive value | Laws (CE n° 258/97)
Ecology | Allergic risk ? Anaphylaxis ? Angioedema?
Low resources | 

**Could be FATAL ?**

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Aims of our study

• Evaluation of the risk of cross-reactions with insect allergens:
  – HDM allergy accounts for 5 to 15% of all allergic patients worldwide
  – Shellfish allergy accounts for 0.5 to 1.5% of all allergic patients worldwide

• Development of new diagnostic tools to point out insect sensitization

• Screening of new foods containing insects ➔ control of labeling
What about allergy?
Epidemiology of allergy

Food allergy:
- 2-3% of world population
- 2-6% of children

Allergic asthma = first chronic disease in childhood

x2 during the last two decades in Europe
Allergy

Definition:
An abnormal reaction of the body to a previously encountered allergen

- Introduced by inhalation, ingestion, injection or by skin contact
- Inadapted and excessive response of our organism
- Reproducible reaction
- Reaction even if there is a low quantity of allergen
- Mainly mediated by IgE (= allergy linked antibody)
Allergy

➢ Sensitization phase:
After an initial contact with an allergen ➔ activation of the immune system

➔ NO SYMPTOMS
➔ Specific IgE

➢ Reaction phase:
Ulterior contact with the same allergen

➔ REACTION (clinical symptoms = allergy)

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Allergy

Sensitization phase (1) (2) (3)
Allergic reaction phase (4) (5)

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Definition:
A substance (mainly protein) that can cause an allergic reaction but is not harmful to most people.

In our case, we studied shrimp and House Dust Mites allergens that are already described and used for *in vitro* diagnostics:

<table>
<thead>
<tr>
<th>Shrimp allergens</th>
<th>HDM allergens</th>
</tr>
</thead>
<tbody>
<tr>
<td>slgE shrimp extract</td>
<td>slgE <em>Dermatophagoïdes pteronyssinus</em></td>
</tr>
<tr>
<td>slgE tropomyosin (rPen a 1)</td>
<td>slgE <em>Dermatophagoïdes farinae</em></td>
</tr>
<tr>
<td></td>
<td>slgE rDer p 1</td>
</tr>
<tr>
<td></td>
<td>slgE rDer p 2</td>
</tr>
<tr>
<td></td>
<td>slgE tropomyosin (rDer p 10)</td>
</tr>
</tbody>
</table>

What about cross-reactivity?
Cross-reactivity

Definition:
Cross-reactivity in allergic reactions occurs when the allergenic proteins in one allergenic source are similar to the proteins found in another source.

Example:
If you are allergic to birch tree pollen, you may have an oral allergy symptom reaction when eating an apple.
Cross-reactivity

Tropomyosin
Major allergen of invertebrates and minor allergen of House Dust Mites (HDM)
Marker of cross-reaction between shellfish, HDM and insects

Can cause severe allergic reactions

Example:
Some of the HDM allergic patients present an allergy when eating shellfish due to cross-reaction
→ only if previously sensitized to tropomyosin!

In vitro:
- rPen m 1
- rDer p 10
- nBla g 7
- rAni s3
- rPen a 1
- nPen i 1

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Diagnosis of allergy in a clinical laboratory
Diagnosis

- Clinical history
- Allergic reaction characterization:
  - *In vivo* assays
    - Skin prick test
    - Oral food challenge
  - *In vitro* assays
    - Measurements of specific IgE in the patient’s serum
      - Traditional method ImmunoCAP250 (ThermoFisher Scientific)
      - ImmunoCAP ISAC (microarray)
R&D allergy diagnosis

- 1D & 2D Western blot
  - Detection of the proteins against which the patient has been sensitized
  - Sensitization profile of one patient!
Diagnosis

1. **Protein extraction**
   - Urea
   - CHAPS
   - TBP
   - Biolytes
   - Total proteins

2. **2DE Electrophoresis**
   - IEF → Electric charge
   - SDS-PAGE → Size
   - pH 3 → pH 10
   - HMW Proteins
   - LMW Proteins

3. **Protein transfer**
   - Cathod
   - Blotting paper
   - Protein
   - SDS-PAGE
   - PVDF membrane
   - Blotting paper
   - Anod

4. **Western blot**
   - Detection signal
     - Colorimetric or chemiluminescent
   - Enzyme-conjugated Secondary Antibody
   - Patient Antibody
   - Target protein
   - Membrane containing transferred protein

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What about insect food allergy?
Insect Food allergy

Major described allergens:
- Tropomyosin
- Arginine kinase
- Chitin...

Cross-reactions with shellfish and/or HDM allergens?

Allergens are not always proteins…
How?

• Total protein extraction from crickets (*Grillodes sigillatus*)

**EITHER**

• SDS-PAGE gel (1D)
• Western blot (1D)

**OR**

• Isoelectric focalization (1D)
• SDS-PAGE gel (2D)
• Western blot 2D
Results:
protein extraction from crickets

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First case analyzed: 1D

27 y.o. woman with shellfish and HDM allergies

Systemic reactions

- SLgE Shrimp = 9.89 kUA/L
- SLgE Shrimp tropomyosin (rPen a 1) = 14.3 kUA/L
- SLgE HDM (rDer p1) = 92.4 kUA/L
- SLgE HDM (rDer p2) >100 kUA/L
- SLgE HDM tropomyosin (rDer p10) = 16.9 kUA/L

Western blot 1D with *Grillodes* extract

Protein of interest (allergen) detected by Western blot 1D

Arginine kinase? Tropomyosin?

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First case analyzed: 2D

Western blot 2D with *Grillodes* extract

Protein of interest (allergen) detected by Western blot 2D

*Insectinov 2 - Adebiotech / AgroParisTech*
Second case analyzed: 2D

30 y.o. woman with HDM allergy

Atopic dermatitis

sIgE Derm.pteron = 66.3 kUA/L
sIgE Derm.farinae = 45.1 kUA/L
sIgE HDM (rDer p1) = 23.4 kUA/L
sIgE HDM (rDer p2) = 37.9 kUA/L
sIgE HDM tropomyosin (rDer p10) = 0.18 kUA/L

Western blot 2D with *Grillodes* extract

Protein of interest (allergen) detected by Western blot 2D

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38 y.o. man with shrimp allergy

Oral allergy syndrome

**slgE shrimps**

= 5.60 kUA/L

**slgE HDM**

(rDer p1)

< 0.1 kUA/L

**slgE HDM tropomyosin**

(rDer p10)

< 0.1 kUA/L

Western blot 2D with *Grillodes* extract

Proteins of interest (allergens) detected by Western blot 2D

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In conclusion
Conclusion: preliminary results

- IgE Cross-reactivities have been shown between shrimp, HDM and crickets
  - Tropomyosin or Arginine Kinase cross-sensitization
  - Sensitization to the *Gryllodes’* troponin C

**New allergens identified by WB 2D**

- The identification of these proteins should be confirmed by mass spectrometry (LC-MS/MS)
- Should be aware of potential allergic reaction in sensitized populations... Should be investigated!

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Thank you

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