Food Allergy

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Food Allergy Research
USDA-ARS-Southern Regional Research Center
The prevalence of food allergy has increased and in some cases doubled since 1997:

- Better diagnosis
- New cases/increased rate of sensitization
- Increased consumption
- Low dose exposure at an early age
- Exposure through unnatural routs
- Any exposure at an early age
- Vaccinations/better Hygiene
- Processing methods/globalization of markets
Food Allergy

Approximately 11 million American suffer from food allergies. The most common being fish/shellfish, peanut and tree nut allergies. Eating foods that contain even traces of these ingredients can lead to health problems ranging from itchiness and skin rashes to anaphylactic shock.
Major Food Allergens

Eight major foods or food groups--milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, and soybeans--account for 90 percent of food allergies (& sesame in Europe)
Recent studies estimate that over 11 million Americans have a food allergy.

Over 6 million are allergic to fish and shellfish alone.

Over 3-4 million are allergic to peanuts and tree nuts and the number of children with peanut allergy has doubled in the past five years.

Each year, over 125 Americans die due to the accidental ingestion of allergenic foods.

30,000 receive life-saving treatment in emergency rooms.
### Why accidental ingestion?
#### Foods that may contain peanut

<table>
<thead>
<tr>
<th>Baked goods</th>
<th>Milk formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baking mixtures</td>
<td>Pastry</td>
</tr>
<tr>
<td>Battered foods</td>
<td>Other nut butters</td>
</tr>
<tr>
<td>Biscuits</td>
<td>Satay sauces/dishes</td>
</tr>
<tr>
<td>Candy</td>
<td>Soups</td>
</tr>
<tr>
<td>Cereal-based products</td>
<td>Sweets</td>
</tr>
<tr>
<td>Canned Chili &amp; Stews</td>
<td>Vegetable fats/oils</td>
</tr>
<tr>
<td>Chinese (egg rolls), Indian, Indonesian Thai, African, etc dishes</td>
<td>Ice cream</td>
</tr>
<tr>
<td>Margarine</td>
<td></td>
</tr>
<tr>
<td>Cookies</td>
<td></td>
</tr>
</tbody>
</table>

Even among the well informed, 55% of allergic patients have **1-2** accidental ingestions over a **5 year** period.
Overall Research goals and Objectives:

- Improve allergen detection and diagnostic tools
- To develop novel therapeutic tools for the treatment of Peanut Allergies. (vaccine, anti-IgE & T-cell immunotherapy, probiotics, functional foods)
- Genetically engineer hypoallergenic plants
- Reduce allergenicity through novel processing methods
- Find peanut varieties with reduced levels of allergens or allergenic properties
anti-IgE

IgE

Histamines

Cytokines

APC

B

M

T

T

T

T

T

T
Collect blood from peanut allergic patient
## The Allergens Identified in Peanut

<table>
<thead>
<tr>
<th>Name</th>
<th>Protein</th>
<th>% in Seed</th>
<th>MW</th>
<th>% Individuals allergic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ara h 1</td>
<td>vicillin</td>
<td>~12%</td>
<td>63 KDa</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>Ara h 2</td>
<td>conglutin</td>
<td>~0.5%</td>
<td>18, 20 KDa</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>Ara h 3/4</td>
<td>glycinin</td>
<td>~25%</td>
<td>60 KDa</td>
<td>~50%</td>
</tr>
<tr>
<td>Ara h 5</td>
<td>profilin</td>
<td>&lt;1%</td>
<td>14 KDa</td>
<td>&lt;20%</td>
</tr>
<tr>
<td>Ara h 6, 7</td>
<td>conglutin homolog</td>
<td>&lt;1%</td>
<td>12-15 KDa</td>
<td>~50%</td>
</tr>
<tr>
<td>Ara h 8</td>
<td>glycinin homolog</td>
<td>&lt;1%</td>
<td>16 KDa</td>
<td>?</td>
</tr>
<tr>
<td>Ara h 9</td>
<td>oleosin</td>
<td>&lt;1%</td>
<td>18 KDa</td>
<td>?</td>
</tr>
</tbody>
</table>
Purification of the major allergens identified in peanut
Purpose for Identification and Purification of Allergens

- Allergen structure function analysis
- Allergen cloning
- Development of diagnostic tests
- Development of detection tests
- Tissue culture/ T-cell, B-cell
- Histamine release/Mast cell, Basophil
- Animal model testing
- Anti-Allergen Antibody Production
- Cross-reactivity analysis
- etc
Ara h 1 forms highly stable trimers through hydrophobic interactions

Ara h 1 forms highly Stable trimers that are resistant to digestion by digestive enzymes:

Anti-Allergen, Antibody Production
Introduction

Different peanuts varieties may have

- different levels of total protein
- different amounts, structure or sequence of individual protein and or allergen
- less of one protein or allergen and more of others

Ideally we want to find or breed a peanut with high protein and low allergen content
Future Possibilities

A peanut with significantly reduced allergens or allergenicity may:

- reduce sensitization capability of peanuts
- increase threshold dose
- result in a less severe reaction upon accidental ingestion
- be useful in immunotherapeutic desensitization
- be more acceptable than a hypoallergenic GMO peanut
Background

- Approximately 300 irradiated NC4 peanuts were screened for the levels of Ara h 1, 2 & 3.

- Peanuts were found that exhibited lower levels of the allergens Ara h 1, Ara h 2, or Ara h 3.

- Peanuts were also found that lacked either an isoform of the allergens Ara h 2 or Ara h 3.
Peanut Cultivars with either a significant reduction in, or missing, isoforms of Ara h 1, Ara h 2 or Ara h 3 have been found:
## Anti- Ara h 1, Ara h 2, and Ara h 3

### Western blots with Mutants of Interest

<table>
<thead>
<tr>
<th></th>
<th>Ara h 1</th>
<th>Ara h 2</th>
<th>Ara h 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutant 1</td>
<td>3 24 159 168 184 280</td>
<td>13 232 8 “Rusty” 217</td>
<td>9 11 15 (NC146)</td>
</tr>
<tr>
<td>Mutant 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutant 3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **High level of Ara h 1**
- **Low level of Ara h 1**
- **Reduced level of Ara h 3**
- **Missing an Ara h 2 isoform**
- **Missing an Ara h 3 Isoform**

*Note: The image shows a Western blot analysis of different Ara h variants in different mutants.*
Next Step

A peanut variety that lacked one of the Ara h 3 isoforms was cross-bred with the one missing an Ara h 2 isoform.
Missing an Ara h 3 isoform

Missing an Ara h 2 isoform
Objective

Screen over 100 of the F2 generation or progeny of crossbred peanuts to ascertain if we created a peanut missing both the Ara h 2 and Ara h 3 isoforms.
Western blot analysis with both anti-Ara h 2 and anti-Ara h 3 antibodies
Western blot analysis using serum IgE from peanut allergic individuals

Differential IgE binding to Ara h 2 and Ara h 6 is seen in the mutant peanuts for the different individuals. This indicates amino acid sequence differences exist among the same allergens in the varieties.
Conclusions

Over 500 peanut varieties from the peanut germplasm collection and approximately 400 irradiated mutants and progeny have been screened by our laboratory so far.

Many differences were found among the screened peanuts such as:
- Allergen levels
- Allergen isoform numbers
- IgG and IgE binding
- Presence of new IgE binding bands
Conclusions

Through traditional breeding it is possible to knockout some of the allergenic proteins in plants, which may ultimately:

- reduce the severity of the allergic response
- reduce sensitization capability
- be useful in immunotherapeutic desensitization
- be useful in understanding valuable genetic information such as the genetic inheritance patterns of the allergens. (i.e. Co-inheritance of the missing isoforms followed the classical Mendelian inheritance pattern of 1:15)

The peanut industry and market are less likely to suffer from boycotts or price fluctuations seen while attempting to market genetically modified organisms (GMO)
Acknowledgements:
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Future Studies

- Perform initial skin prick tests with the current mutants lacking a single isoform or both Ara h 2 and Ara h 3 isoforms and mutants with reduced overall IgE binding capacity
- Plant and grow the seeds identified to lack both an Ara h 2 and an Ara h 3 isoform
- Crossbreed the peanuts that lacked both isoforms with peanuts that showed reduced levels of Ara h 1
- Cross breed the current progeny that are missing both isoforms with peanuts found to be missing other allergenic proteins or currently marketed peanuts
- Begin screening mutants and other varieties with anti-Ara h 6 antibody