Peanut Allergy Research

Peanut Allergy Research Unit
USDA-ARS-New Orleans, LA
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Ongoing Research

- Improve **diagnostic/detection** methods (threshold dose, detection kit, allergen identification, purification and characterization, standard extracts, cross-reactivity, etc)

- Determine the **threshold dose** and the effects of processing on threshold dose, epidemiology and sensitization.

- To develop **novel therapeutic** tools for the treatment of peanut allergies. (vaccine, anti-IgE, peptide, cytokine, APC & T-cell immunotherapy)

- Genetically engineer **hypoallergenic plants** (gene silencing, mutation, replacement, knock out)

- Find **peanut varieties** with **naturally** reduced levels of allergens or allergenic properties (screening with antibodies)
Sensitization & skin test reactions of peanuts processed and ingested in different forms
Mouse model for comparing sensitization
(USDA, LA & Japan)

What happens to peanuts following different processes?
**Solubility of peanut proteins following different thermal processes**

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Raw</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Fried</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Roasted</td>
<td>9</td>
<td>21</td>
</tr>
</tbody>
</table>

Protein solubility is decreased with increased exposure to thermal treatment.

*SDS-PAGE*
Effect of thermal processing on IgE binding

IgE Western

<table>
<thead>
<tr>
<th></th>
<th>Boiled</th>
<th>Fried</th>
<th>Roasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>R Sol.</td>
<td>5</td>
<td>2.5</td>
<td>9</td>
</tr>
<tr>
<td>R Insol.</td>
<td>15</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>R R</td>
<td>5</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>S Sol.</td>
<td>45</td>
<td>5</td>
<td>52</td>
</tr>
<tr>
<td>S Insol.</td>
<td>15</td>
<td>1</td>
<td>21</td>
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<tr>
<td>S R</td>
<td>45</td>
<td>5</td>
<td>52</td>
</tr>
<tr>
<td>S S</td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

R = Raw Extract; S = Soluble Fraction; I = Insoluble Fraction

IgE binding is reduced in the soluble fraction as exposure time increases.
IgE binding to trypsin & pepsin digested, differently processed peanuts

IgE binding to peanut allergens is increased as exposure to heat is increased
Some facts about the peanut allergens

Nine allergens have been identified in raw peanut.

Five of these allergens (Ara h 1,2,3,4 & 6) have been purified from raw and 3 of them Ara h 1, 2, 3 & 4) from roasted peanut.

The IgE binding sites for Ara h 1, 2, 3 & 4 (the major allergens) have been identified.
Some facts about the peanut allergens

- The cDNA for all of the 9 allergens have been cloned.

- The cDNA’s for Ara h 1, 2 & 3/4 have been altered to eliminate or reduce IgE binding significantly (hypoallergen).

- The genomic clones for Ara h 1, 2, 3 & 4 have been identified.
Identification and Purification of the Major Peanut Allergens & Anti-Allergen, Antibody Production
# 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>~1%</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>14 KDa</td>
<td></td>
<td>&lt;20%</td>
</tr>
<tr>
<td>Ara h 6, 7</td>
<td>conglutin homolog</td>
<td>~1% 15, 17 KDa</td>
<td>&gt;50%</td>
<td></td>
</tr>
<tr>
<td>Ara h 8</td>
<td>glycinin homolog</td>
<td>16 KDa</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Ara h 9</td>
<td>oleosin</td>
<td>18 KDa</td>
<td></td>
<td>?</td>
</tr>
</tbody>
</table>
Purification of the major allergens identified in peanut

Ara h 2           Ara h 1     Ara h 3,4   Ara h 6
Purpose of Identification and Purification of the Major Peanut Allergens

- Allergen structure function analysis
- Allergen cloning
- Development of diagnostic tests
- Tissue culture: T-cell, B-cell
- Histamine release/Mast cell, Basophil
- Animal model testing
- Anti-Allergen Antibody Production
- Cross-reactivity analysis etc
Structure, Biophysical Properties and IgE Binding to the Major Peanut Allergens (i.e. allergens are classically thought to be resistant to digestion)
Fluorescence Polarization Analysis of Ara h 1

Ara h 1

mP

[10 100 1000] [Ara h 1] nM

Ara h 1 forms trimers
Ara h 1 forms highly stable trimers that protect IgE binding sites from digestive enzymes.

Maleki et al., (June, 2000) *J. Immunology*, 164: 5844-49
Ara h 2 has sequence homology to trypsin inhibitors
## Trypsin inhibitory activity of Ara h 2 from roasted vs raw peanuts

<table>
<thead>
<tr>
<th>Sample</th>
<th>Trypsin inhibitor Activity (unit/ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ara h 2 from Raw Peanuts</td>
<td>21.38</td>
</tr>
<tr>
<td>Denatured Ara h 2</td>
<td>68.2</td>
</tr>
<tr>
<td>Ara h 2 from Roasted Peanuts</td>
<td>74.66</td>
</tr>
</tbody>
</table>
CD spectrum of Native Ara h 2:

Raw: ~60% α-helix, ~3% Beta sheet, 37% Random Coil
Roasted: ~58 α-helix, ~4% Beta sheet, 38% Random Coil
Ara h 2, a digestive enzyme inhibitor

Trypsin binding loop residues 29-36

Threshold dose  
(Multicenter project)

Kinetics of absorption  
(USDA, LA& MD)

Fragments of allergens that survive digestion /activated Charcoal  
(USDA, MD/St.Michaels)

Sensitization ability of the surviving fragments (USDA, LA, Canada, UAMS, Japan).
Secretion of Peanut Protein in Saliva following ingestion
Detecting Ara h 1 in breast milk of a non-allergic volunteer (same person, 2 ELISAs)
Why a food should be studied in the form that it is ingested

- The Structure/Function of Peanut Proteins
  - *digestion* in the gut
  - *absorption* into the blood stream
  - IgE binding
  - histamine release
  - T cell proliferation
  - threshold dose/sensitization

- Age and frequency of *consumption* by infants may influence the *Epidemiology* of peanut allergy.
Screening Peanut Cultivars for Reduced levels of Allergens

(USDA: LA, NCSU)

Funded by the Georgia Peanut Commission, The Peanut Foundation & USDA
Peanut Cultivars missing Ara h 1, Ara h 2 and Ara h 3 have been found: (USDA, NCSU)

These varieties are currently being crossbred at NCSU to produce reduced/hypoallergenic peanuts.
Anti-Ara h 1, Ara h 2, and Ara h 3 Western Blot on peanut varieties of Interest
Missing an Ara h 3 isoform

Missing an Ara h 2 isoform

NC Ac 01460

“Rusty”
Western blot analysis with both anti-Ara h 2 and anti-Ara h 3 antibodies

Ara h 2 isoforms

Ara h 3 isoforms

Ara h 2 isoforms

Ara h 3 isoforms
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 is less likely to suffer from boycotts or price fluctuations seen while attempting to market genetically modified organisms (GMO)
Other USDA projects not discussed:

- **Immunotherapy:**
  - Identification of T cell epitopes
  - T cell signal transduction/cytokine secretion
  - Computer modeling of common IgE binding sites
  - APC driven T cell inactivation
  - IgE epitope mapping of Ara h 5-8
- **Natural History/sensitization:**
  - Does peanut protein in breast milk sensitize or tolerize infants
- **Epidemiology**
  - Characterization and comparison of the allergenicity of legume based foods from within and outside of the USA.
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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.
Cross sensitization of mice with raw and roasted peanuts

Antibody titers in mice sensitized with raw and roasted peanuts