Evolution of the Allergy Assessment Process

IFBic and HESI Biotechnology Update

September 16, 2010
Allergy Assessment

- **Allergy**
  - Diagnosis is difficult
  - Perceived vs actual allergy rates
  - Allergy methods are evolving
  - No general monitoring of food allergy
  - Many stakeholders
  - Emotional topic
Allergy Assessment

• Three categories of public health risk regarding potential protein allergenicity and biotechnology:
  
  ➢ Transfer of known allergens or cross-reactive proteins
  
  ➢ Increasing endogenous allergenicity of an already allergenic crop
  
  ➢ Novel proteins becoming allergens in sensitive populations

*No scientific evidence that a biotech protein or a GM crop increased allergenic risk to the susceptible public*
Allergy Assessment

• Allergy Assessment Goals for GM Products
  – Determine the allergenic potential of the introduced protein
  – Determine endogenous allergenicity of GM crop vs conventional control

• 8 foods account for >90% of food allergic reactions

<table>
<thead>
<tr>
<th>Milk</th>
<th>Wheat</th>
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<tbody>
<tr>
<td>Eggs</td>
<td>Peanuts</td>
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<tr>
<td>Fish</td>
<td>Soybeans</td>
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<tr>
<td>Crustacea</td>
<td>Tree nuts</td>
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</tbody>
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Disease management by avoidance (elimination diets)
Allergy Assessment

• Based on evolving international guidelines (1996-Present; ILSI, Codex, EFSA, Japan, Korea, Taiwan)

• Determine the allergenic potential of the introduced protein
  – Source organism
  – Similarity to known allergens
  – Digestive fate
  – Serum study, if appropriate

• Determine the endogenous allergenicity of GM crop vs conventional control and references
  – Patient sera from clinically documented, appropriately allergic patients
Allergy Assessment

• **RR1 (1996)**
  - Source organism
    - Literature search
  - Similarity to known allergens
    - Monsanto allergen database
    - FASTA/sliding window of introduced protein
    - No established cut-offs
  - Digestive fate
    - SGF
    - SIF
    - No standardized assay conditions

• **RR2Y (2006)**
  - Source organism
    - Literature search
  - Similarity to known allergens
    - International allergen database
    - FASTA/sliding window of entire T-DNA (all frames), genomic flanks
    - Cut-offs of 35%-80aa identity/8aa sliding window
  - Digestive fate
    - SGF
    - SIF
    - **Standardized assay conditions**
Allergy Assessment

• **RR1 (1996)**
  - Endogenous allergenicity of GM product
    • Pooled sera from 5 soy allergic patients
    • Compared to 4 soy controls
    • 1D Western blot

• **RR2Y (2006)**
  - Endogenous allergenicity of GM product
    • Individual sera from 16 soy allergic patients
    • Compared to 24 Soy controls
    • Quantitative ELISA/2D western blots
Allergy Assessment

• International Allergen Database
  – Q: “What assurances can we give that all allergens are contained in the allergen database?”
  – Industry led effort to establish AllergenOnline
    • All major Ag Biotech companies fund the database (Monsanto, Syngenta, DuPont, Dow, Bayer, BASF)
    • Maintained by the University of Nebraska
    • Criteria established for inclusion into the database
    • Curated by panel of internationally recognized allergists
    • Update yearly
Allergy Assessment

• FASTA/sliding window of entire DNA (all frames), genomic flanks
  – Q: “Are there any ORFs in the T-DNA or in the genomic DNA flanking the T-DNA that encode allergens?”
  – Established comprehensive bioinformatic analyses and report that takes into account:
    • All 6 frames of the entire T-DNA
    • The insert/genomic DNA junctions
    • Genomic DNA flanking the T-DNA
Protein sequence bioinformatics reports

Schematic of *in planta* DNA sequence

- **Flank**
  - **T-DNA**
  - **Flank**

**POI coding sequence**

**RR1**
- POI coding sequence
  - Frame 1, FASTA (AD, TOX, PRT databases) and 8-mer (AD database)

**RR2Y**
- Entire T-DNA sequence
  - Frames 1-6, FASTA (AD, TOX, PRT databases) and 8-mer (AD database)

**Flank Junctions**
- Frames 1-6, FASTA (AD, TOX, PRT databases) and 8-mer (AD database)
Allergy Assessment

• Cut-offs of 35% identity/8aa
  – Q: “How can we be sure that the cut-offs used for allergen bioinformatics analyses are valid?”
  – 35% identity over 80 amino acids or greater:
    • Based on recommendation of FAO 2001 (Bet v 1)
    • Work by many have shown that this cut-off is very conservative and results in many false positives
    • Combination of 35% identity over 80 amino acids and e-scores improves the efficiency and accuracy of identifying cross-reactive allergens
  – 8aa sliding window
    • FAO 2001 recommended 6 aa
    • Work by many have shown that use of a 6 aa sliding window identifies proteins based on chance not allergen characteristics
    • Successfully removed 6aa sliding window from the conversation
Allergy Assessment

• Digestive Fate – Standardized Assay Conditions
  – Q: “Why do your SGF assay conditions differ from others?”

  • ILSI sponsored “ring” test to establish common assay conditions for regulatory studies
  • “Ring” test participants included industry, academics, and regulators
  • Established a standardized SGF assay
Allergy Assessment

• **Endogenous Allergenicity**
  
  – “We want a quantitative and qualitative assessment of endogenous allergenicity”

1. MWM
2. 61-67-1
3. A5403
4. 40-3-2
5. Cargill 1
6. Cargill 2
7. ADM

RR1 VS RR2Y
Allergy Assessment

• Future challenges for the allergy assessment process
  • Logistics and ethical questions surrounding the use of appropriately allergic patient sera
  • Regulator’s desire for a quantitative assessment of individual allergens
  • “Omics” technologies
Allergy Assessment

• ILSI Sponsored Workshops and Publications Support the Scientific Basis for the Allergy Assessment Process

General Allergy

Similarity to Known Allergens

Digestive Fate/Stability

Serum Studies

Endogenous Allergenicity