Safety of GM Food Crops: Protein Allergenicity Assessment

Nairobi, Kenya 13th August, 2014

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General Considerations - Proteins

Proteins and the human diet

- Macronutrients: protein, fat, carbohydrate, fiber
- Exposure is relatively “High” - Essential component of the human diet
  - 100 g/day consumed
- Most dietary proteins are not absorbed intact but are digested and exposure is not to the fully functional protein
- Protein consumption is not inherently associated with adverse effects
- Only a small number of proteins are known to be allergenic
Safety - A Weight of Evidence Approach

Interpretation of study results are taken into consideration using a holistic approach – all studies are used to perform a risk assessment.

Risk = hazard multiplied by exposure

● If no adverse findings: the protein has “reasonable certainty of no harm”

● When an uncertain finding for any one test is found:
  - Could indicate that the protein represents a hazard
    - (E.g. – shares some characteristic with known allergens)
  - *Further testing* would be indicated and necessary to demonstrate safety or potential allergy
Allergy Health Risks - Biotech Proteins

- Has the protein been *unintentionally transferred* from a known allergen source and is the protein itself a known allergen? Is the novel GM protein *cross-reactive* with known allergens.

- Will the novel protein expressed in the biotech product become an allergen once exposed to workers/consumers? *i.e.*, is there risk of a *de novo* allergen?

- Has the transformation *process increased the normal expression of endogenous allergens* in such a way to increase the risk to allergic patients?
Cellular Basis for the Formation of Type I, IgE Mediated Allergic Responses

Antigen Presenting Cell  \(\rightarrow\) allergen  \(\rightarrow\) B-cell  \(\rightarrow\) IgE  \(\rightarrow\) Mast cells, Basophils  \(\rightarrow\) Mediators; histamine

Clinical Effects:
- asthma/wheezing
- urticaria/rash
- nausea/vomiting
- anaphylactic shock

Characterize the similarity between allergens and novel proteins
no similarity = low risk of developing the biological basis of allergy.

Interleukins: IL-4, IL-13
Allergy Health Risks - Biotech Proteins

Allergy Risk Characterization

- Gene from allergenic source?
- Is sequence similar to known allergens/toxins?
- Is pepsin digestibility rapid?
- Glycosylated?
- Exposure likely after heat/processing?
- Is Abundance low?
- Does protein have a history of safe exposure?

Evidence Supports Allergy Safety

Risk Level

Higher

Low
Allergy and Biotechnology: Safety Guidance

*Evolving allergy strategy to manage health risks*

- **CODEX**
  - Intergovernmental body (>180 member states)
  - Implements joint FAO/WHO Food standards programs
  - Protects health of consumers and facilitates trade by setting international safety standards
  - *No single test can predict protein allergenicity*
Allergy and Biotechnology: Codex Guidance

**If introduced protein from a non-allergenic source**
- assess in vitro pepsin resistance by using a standardized protocol (Thomas et al., 2004, Regul. Toxicol. Pharmacol., 39:87-98)

**If introduced protein from an allergenic source**
- assess amino acid sequence similarity to known allergens (bioinformatics)
- assess in vitro pepsin resistance
- assess specific IgE binding, when applicable
Adaptive allergy strategy to manage health risks

• CODEX recommended allergy assessment
  ➢ Other considerations
    ✓ Exposure level of the introduced protein
    ✓ As science and technology evolves other methods may be considered

  ➢ Targeted serum screens
  ➢ Animal models
  ➢ T-cell epitopes, structural motifs associated with allergens
Allergy and Biotechnology: Allergy Safety

**Adaptive allergy strategy to manage health risks**

- Science of allergy is still evolving
- Current assessment process utilizes best science available
- Current assessment process is successful at preventing the introduction of known or cross-reactive allergens into the food supply
- Harmonization of the allergy assessment process is underway
- Safety process utilizes a “weight of evidence” approach;
  - the goal is to add to this approach with scientifically justified methods, when appropriate.
What’s the Missing Piece?

The current allergy assessment process is useful and robust for novel protein allergy assessments.

- However, We are still missing a true... Model

- New methods are encouraged if they:
  - Are scientifically justified
  - Lend value to the allergy safety assessment process

- In vitro cell assays, animal models, and proteomics are evolving techniques that require validation in allergy assessments.
Bioinformatics - Standardized Approaches for Allergy Risk

- Focused on one primary question:
  - Is the query (novel) protein similar to an existing allergen?
  
  Can also use the results to identify source organism of a trait protein.

- Screening based on knowing the sequences of hundreds of food and respiratory protein allergens

- Identifying significant similarity and possible homology with known allergens means that a novel protein may cross-react with an existing allergen.

- However, bioinformatics is not intended to predict whether a protein will “become” an allergen.

- Bioinformatics is rarely an answer in and of itself.
Bioinformatics - Principles

- Protein structure is determined by amino acid sequence
- Similar amino acid linear sequences have similar structure/function
  - Similar sequence and structure infers a common ancestor and related function across species (Taxonomic relatedness)
- Allergen homologues (highly similar proteins) from two species are often allergens, but not always
- Non-homologous proteins do not cross-react
Bioinformatics - *comparing one-to-many*- Is there a match?

GM Protein Sequence

...ARYQPERSSTR...

Qualified Database of Allergen Sequences

...ARYQPERSSTR...

...SEQQTMGFTA...

...MTYYQSDVEKET...

...FYVEQDSDEVYY...

GM sequence is compared in a one-to-many alignment process

Sequences may not match at all, or match so little, that the process does not report them

Homologous match!

Database sequences that match the *Query* are judged on the significance of the match

...ARYQPERSSTR...

...ARYQPERSSTR...
Bioinformatics - Where does it play a role in safety?

● Bioinformatics plays a continual role throughout a novel protein’s development life cycle; this is because -

  - Bioinformatic reviews can be updated frequently

  - New allergens and toxins are discovered which creates an update to the databases used for bioinformatics

● Helps to inform other aspects of the protein safety assessment

  - HOSU: similarity with proteins with safe records

  - Mode-of-action
Critical to any kind of bioinformatics - a well understood database

- Industry consortium database at University of Nebraska
  - Leaders in the field, Rick Goodman and Steve Taylor host a peer-reviewed allergen sequence database

- What is it?
  - A database of 1,706 whole or partial protein sequences with a minimum of at least one publication supporting IgE binding or other clinical evidence and the sequence itself as a relevant human allergen.
Simulated Gastric Fluid: protein stability to pepsin enzyme proteolysis

- Characterizes the rate at which a protein is degraded:
  - Interpretation: if a novel protein is “stable”, i.e., as stable as some allergens, then risk of exposure and therefore, the potential for allergy is greater.

- Method standardization has been key for screening novel food proteins (Thomas et al, 2004 – An ILSI/PATC publication); regulators are able to compare results from one company to the next for similar proteins.

- The characterization of simulated gastrointestinal stability for novel proteins has expanded to include an additional characterization.
  - Simulated Intestinal Fluid assay (pancreatic enzyme mix) can be included in assessments of novel proteins.
### Table 1. Summary of allergen and protein stability in SGF.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Stability (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole Protein</td>
</tr>
<tr>
<td><strong>Egg allergens</strong></td>
<td></td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>60</td>
</tr>
<tr>
<td>Phosvitin</td>
<td>60</td>
</tr>
<tr>
<td>Ovomucoid</td>
<td>8</td>
</tr>
<tr>
<td>Conalbumin</td>
<td>0</td>
</tr>
<tr>
<td><strong>Milk allergens</strong></td>
<td></td>
</tr>
<tr>
<td>β-lactoglobulin</td>
<td>60</td>
</tr>
<tr>
<td>Casein</td>
<td>2</td>
</tr>
<tr>
<td>BSA</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Soybean allergens</strong></td>
<td></td>
</tr>
<tr>
<td>β-conglycinin (β-subunit)</td>
<td>60</td>
</tr>
<tr>
<td>SKTI</td>
<td>60</td>
</tr>
<tr>
<td>Soy lectin</td>
<td>15</td>
</tr>
<tr>
<td>β-conglycinin (α-subunit)</td>
<td>2</td>
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<tr>
<td>Gly m 1</td>
<td>0.5</td>
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<tr>
<td><strong>Mustard allergens</strong></td>
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<tr>
<td>Sin a 1</td>
<td>60</td>
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<tr>
<td>Bra j IE</td>
<td>60</td>
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<tr>
<td><strong>Peanut allergens</strong></td>
<td></td>
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<tr>
<td>Ara h2</td>
<td>60</td>
</tr>
<tr>
<td>Peanut lectin</td>
<td>8</td>
</tr>
<tr>
<td><strong>Common plant proteins</strong></td>
<td></td>
</tr>
<tr>
<td>Glycine reductase (spinach leaf)</td>
<td>0.25 (15 sec)</td>
</tr>
<tr>
<td>Rubisco LSU (spinach leaf)</td>
<td>0 (&lt;15 sec)</td>
</tr>
<tr>
<td>Rubisco SSU (spinach leaf)</td>
<td>0 (&lt;15 sec)</td>
</tr>
<tr>
<td>Lipoxygenase (soybean seed)</td>
<td>0 (&lt;15 sec)</td>
</tr>
<tr>
<td>PEP carboxylase (corn kernel)</td>
<td>0 (&lt;15 sec)</td>
</tr>
<tr>
<td>Sucrose synthetase (wheat kernel)</td>
<td>0 (&lt;15 sec)</td>
</tr>
<tr>
<td>β-amylose (barley kernel)</td>
<td>0 (&lt;15 sec)</td>
</tr>
<tr>
<td>Acid phosphatase (potato tuber)</td>
<td>0 (&lt;15 sec)</td>
</tr>
<tr>
<td>Phosphofructokinase (potato tuber)</td>
<td>0 (&lt;15 sec)</td>
</tr>
</tbody>
</table>

Expectation for Novel GM Crop Proteins is ~ 2 - 5 min

Astwood, Leach and Fuchs, 1996
Glycosylation

**Glycosylation** – evidence of secondary post-translational modification

- **Is not** consistently associated with clinically relevant allergenic potential

- **Industry**: characterization is by a **standardized in vitro kit** to measure presence of carbohydrate moieties on plant-extracted novel proteins.

- Does not have the priority status of SGF and bioinformatics, but is unlikely to be removed from registrations (and expectations).
  - *Does provide a conservative approach and does add to the weight of evidence for novel protein not undergoing unintended changes, in planta.*
Concluding an Assessment of Allergy Safety

Evidence Does Support Allergy Safety

**Allergy Risk**

- Gene from allergenic source?
- Is sequence similar to known allergens?
  - Is pepsin digestibility similar to allergens?
- Glycosylated?
- Exposure likely after heat/processing?
- Is Abundance low?
- Does protein have a history of safe exposure?

Higher → More applicable to toxicity assessment

Low abundant proteins = lower exposure

Taking on more prominence.

If a protein is derived from a commonly consumed safe food then it should still be safe
Weighing results from tests with imperfect correlations (Codex 2003)

- Reliable prevalence data
  - Proof of allergic responses
- Quality of database
  - Proof of allergenicity
- Additional factors:
  - Abundance in food
  - Stability in heat/processing
- Serum donors: clinical proof
  - Tests: specific inhibition

Accept | Label or Reject

from R. Goodman (Nature Biotech 2008)
Areas of Advancement: Allergy Assessment Methods

How can we measure allergy potential of novel proteins and how can we measure and better characterize known allergens?

- **Bioinformatics**: to address novel protein similarity to known allergens
- Serum screening, when appropriate; *measure cross-reactivity*
- Animal Models; *measure novel proteins or known allergens*
- Proteomics; *characterize and measure concentrations of known allergens*
- New approaches to determining epitopes: *in-depth characterization of known allergens with potential application to novel proteins*
- Cell-based in vitro assays; *assess known allergens - develop test platform for predictive allergy of novel proteins*
Thank you for your attention!
References


