Allergenic Food Proteins (Protein Allergenicity)

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Definitions: Adverse Reactions to Food

**Food Intolerance:**
Non-immune mediated and often associated with food borne substances other than proteins. (enzymatic/metabolic; toxic/pharmacologic)

**Food Allergy:**
An immunologically-mediated response to a food protein that results in allergic sensitization, and upon subsequent exposure, in local or systemic hypersensitivity reactions.
Food Allergic Disease Development

Involves both genetic & environmental factors

Genetic factors

➢ Atopy- inherited tendency to develop IgE-mediated immune responses

➢ Family history found to be most important factor predisposing a child to allergic disease*

 ▪ Atopic history for one parent (50%)
 ▪ Atopic history for both parents (66%)

*Dewar and Wheatley, 1996, Monographs Allergy, 33:4-34
Food Allergic Disease Development

• Environmental factors
  ➢ Protein allergen exposure

• Other factors??
  ➢ Western lifestyle; small family; use of antibiotics; passive smoke exposure
Factors Involved in Development of IgE-Mediated Food Allergy (Protein Allergenicity)

- Genetics
- Exposure to allergenic food
- Age at exposure
- Dose, frequency, and duration of exposure to food proteins
- Immunogenicity of the food
- Development of immunological tolerance
IgE-Mediated Food Allergy Reactions

• Most studied and defined food reactions

• Occur when allergenic food proteins sensitize the gut mucosal immune system, inhalation, skin contact

• Symptoms typically occur within minutes to a few hours (strong temporal relationship)

• Symptoms may be limited to local areas (i.e. oropharynx/gastrointestinal tract), other areas (e.g., skin, airways, eyes), or systemic
Type 1: IgE mediated allergy

Immediate hypersensitivity reactions

- APC
- T-Helper
- B-cell
- IL-4, IL-13

Clinical Effects
- Mast cells
- Basophils
- Mediators
- Histamine
- Heparin
- Leukotrienes
- Cytokines
- Enzymes
Properties of Allergenic Food Proteins

• Retain sufficient structural integrity to be taken up by the gut and sensitize the mucosal immune system.

• Endure the conditions of the GI tract: low pH, proteolytic enzymes, surfactants, and bile salts.

• Allergenicity may be linked to the ability of a protein to pass through gut epithelial barrier and to cross membranes.

• Majority of plant and animal allergenic food proteins share two properties: *Abundance and structural stability*
Common Properties of Allergenic Food Proteins

- Stimulate an immune response (IgG and IgE)
- Generally major food proteins
- Generally resistant to heat and enzymatic digestion
- Multiple disulfide bonds
- Glycosylated
- MW between 10-70 kDa.
Food-Pollen (Oral) Allergy Syndrome

- Associated with the ingestion of raw fruits and vegetables
- Heat labile proteins: cooking OK
- Observed in individuals with seasonal allergic rhinitis due to cross-reactivity between pollens and foods

Birch pollen - apple, carrot, hazelnut, potato, pear, cherry, plum, apricot
Ragweed pollen - banana, melons
Mugwort pollen - celery, kiwi, apple
Grass - cherry, tomato, potato
Food-Pollen (Oral) Allergy Syndrome

- Very rapid onset
- Confined to oropharynx
- Symptoms: pruritus and edema of lips, tongue, palate, throat

Food-Latex Allergy Syndrome- banana, kiwi, avocado, chestnut
Food Allergy Conclusions

• Affect a small percentage of the population

• Reactions can occasionally be severe

• Management of ‘true’ food allergies
  ➢ Specific avoidance diets
  ➢ Epinephrine, self-injectable
  ➢ Symptomatic reactivity to some food allergens lost over time (cow’s milk, hen’s egg)
Agricultural Biotechnology

• Provides a more accurate and precise method of enhancing plant traits

• The selection of a specific genetic trait from a plant or other organism and insertion into the genetic code of another plant

Insect or herbicide resistance; functional foods
Categories of Potential Health Risks Relative to Protein Allergenicity (in order of potential risk)

- Transfer an existing allergen or cross-reactive protein into another crop.
- Creation of food allergens *de novo* (*i.e.*, potential to become a new allergen.)
- Alteration or quantitative increase of endogenous (existing) allergens (*i.e.*, increasing the hazard of currently allergenic foods)
How do we know agricultural biotechnology products are safe?

• There is a comprehensive safety assessment program!
Product Safety Assessment

- Allergenicity
- Toxicology (mammalian)
- Environmental
- Insect Resistance Management

   - Human Dietary Exposure Assessment
   - Gene Flow
   - Environmental Fate/Exposure
   - Ecotoxicology
Protein Allergy Assessment
Holistic Approach

Comparison of the GM crop to a conventional equivalent with a History of Safe Use (HOSU) guides the safety assessment.
Safety Assessment Approach-Protein Allergy

- Avoid transfer of known allergens
  - Assume genes from allergenic sources encode an allergen until proven otherwise
- All introduced proteins evaluated
- Endpoints
  - gene source, sequence homology with known allergens, immunologic analyses and physicochemical properties
- ‘Weight-of-the-evidence’ provides reasonable assurance that foods will not become more allergenic

Metcalf et al., 1996; FAO/WHO, 2001; CODEX 2009
Potential for Protein Allergenicity

Weight of Evidence Approach

• Source of gene(s) / Crop
• Structural features of protein
  – Amino acid sequence comparisons
• Biochemical / biophysical characteristics
  – stability
  – post-translational effects
• Abundance in crop / food
• Sera screening; Animal models?
Categories of Potential Health Risks Relative to Protein Allergenicity

Potential Risk:

1. Transfer an existing allergen or cross-reactive protein into another crop
2. Alteration or quantitative increase of endogenous (existing) allergens
3. Creation of food allergens *de novo*

Technology to assess potential risk per CODEX (2009):

1. **Bioinformatics**/Immuno methods
2. Immuno methods
3. Physical properties of protein (e.g., pepsin stability)
Bioinformatics

• Definition:
  ➢ “the comparative analysis of protein sequences intended to evaluate structural and functional relationships”

• Principles:
  ➢ Protein structure is determined by amino acid sequence
  ➢ Similar amino acid sequences have similar structure
  ➢ Similar sequence and structure infers a common ancestor gene and related function
How does bioinformatics help?

• Allows one *primary* question to be asked:
  Is the protein an existing allergen?

• Allows one *secondary* question to be asked:
  Is the protein likely to cross-react with an existing allergen?

• Bioinformatics is not intended to answer whether a protein will “become” an allergen
Amino Acid Sequence Analysis (Bioinformatics)

• Recommended for all introduced proteins

• Amino acid sequences of allergens in public databases (GenBank; SwissProt) used to construct allergen databases (www.allergenonline.org)

• Evaluate sequence identity for structural relationship and potential epitope matches using local alignment programs, such as FASTA.
  - ≥ 35% identity over 80 or greater amino acids; 8 contiguous identical amino acids
# Categories of Potential Health Risks Relative to Protein Allergenicity

## Potential Risk:

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Specific IgE Sera Screening

• For proteins originating from an allergenic source, or having significant homology with a known allergen, specific serum screening is conducted.

• An issue of critical importance to sera screening is the availability of well characterized, quality human sera from a sufficient number of patients.

• Potential false positives/equivocal results
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Technology to assess potential risk per CODEX (2009):

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3. Physical properties of protein (e.g., pepsin stability)
In Vitro Pepsin Resistance

• Evaluated in simulated gastric fluid (pH 1.2) containing 0.3% (w/v) pepsin.

• Digestions performed for various time intervals 0 - 60 min at 37°C.

• Samples analyzed by SDS PAGE and/or Western blot analysis.

• A standardized protocol for evaluating the in vitro pepsin resistance of proteins was established (Thomas et al., 2004).

Although a correlation between pepsin resistance and allergenic potential has been proposed, the relationship is not absolute.
Conclusions

• Probability of an introduced protein being an allergen is extremely low

• Definitive methods are in place to detect the transfer of known or putative allergens

• Use “weight of evidence approach” in determining the allergenic potential of foods derived from biotechnology
No scientific evidence a commercial GE Crop (or proteins expressed in these crops) has increased potential allergenic risk to the susceptible public
