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Prevalence of Food Allergy

Prevalence of IgE antibody-mediated food allergies among the general population-

1-2% of adults
4-6% of children

6-7 million (U.S)

Public Perception: 30%
Common Allergenic Foods

Eight foods or food groups account for over 90% of food allergies
(peanuts, soybeans, cow’s milk, hen’s egg, fish, crustacean, wheat, and tree nuts)

Prevalence to allergy varies geographically
• Buckwheat and rice allergy: Asia
• Fish allergy: Scandinavia
• Walnut/pecan: U.S.
• Hazelnut: Europe
• Fruit allergy: Spain
“Emerging”: avocado/kiwi; sesame seeds; spices
What Are The Protein Allergenicity Concerns with Biotechnology?
Categories of Potential Health Risks Relative to Allergenicity

1. Transfer an existing allergen or cross-reactive protein into another crop.

2. Alteration or quantitative increase of endogenous (existing) allergens
   (i.e., increasing the hazard of currently allergenic foods)

3. Creation of food allergens de novo
   (i.e., potential to become a new allergen.)
1996 IFBC/ILSI Decision-Tree

- ≥ 8 contiguous identical amino acids
- In vivo clinical testing

FAO/WHO 2001

- ≥ 6 contiguous identical amino acids
- > 35%/80 amino acids
- Animal models
- Targeted sera screening
- No in vivo clinical testing

**Source of gene allergenic**

- **Sequence Homology**
  - Yes
  - No

- **Specific Serum Screen**
  - Yes
  - No

- **Targeted Serum Screens**
  - Yes
  - No

**Pepsin Resistance & Animal Models**

- +/+ High Probability of Allergenicity
- +/- Low Probability of Allergenicity
- +/- No
- -/- No
6 contiguous amino acids

  - Used 6 aa and 8 aa search of corn proteins to determine % of total that matched allergens
    - 82% of corn proteins “matched” allergens on 6 aa search
- Stadler and Stadler, 2003, FASEB J. 17:1141-1143
  - 67% of Swiss-Prot sequences are “allergens” by 6 aa criteria

The use of a 6 aa sliding window search yields an unacceptably high number of false positives and does not provide any useful information in terms of regulatory decisions.
FAO/WHO 2001 Inspired Activities

  - Examined “The Value of Short Amino Acid Sequence Matches for Prediction of Protein Allergenicity”
  - Conducted a series of analyses and calculated match probabilities between a peptide sequence derived from a query protein and a sequence from a protein allergen.
  - Identification of short amino acid sequence matches (e.g., 6) using a sliding window is a product of random chance.

  - Universal agreement - 6 contiguous identical amino acid searches were declared to lack utility in predicting protein cross-reactivity; some debate on utility of sliding window search in general

*The use of a 6 aa sliding window search yields an unacceptably high number of false positives and does not provide any useful information in terms of regulatory decisions.*
> 35% similarity over 80 amino acid window


- Conventional FASTA analysis (overall sequence alignments) produced fewer false positive findings and equivalent false negative rates; generally more significant $E$ scores; a more relevant identity to the query protein; and better reflected functional similarity compared to the 80 amino acid search (Ladics et al., 2007, Mol. Nutr. Food Res. 51:985-998).
Animal models for predicting protein allergenicity

- Active area of research (rodents, dogs, pigs)
- Definite need for further evaluation
  - assay selectivity
  - assay sensitivity
  - broad testing with a range of proteins
- Presently, no animal models (rodent or non-rodent) have been validated or are widely accepted.

Thomas, et al., 2005 meeting poster, AAAAI. Multi-lab study with various mouse strains and several purified allergenic and non-allergenic proteins. Responses of allergenic proteins were similar or less than those of the putative non-allergenic proteins.
AdHoc Intergovernmental Task Force on Foods Derived from Biotechnology

Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants, including Annex: Assessment of Possible Allergenicity.

- CODEX recommended allergy assessment includes:
  - Source of the introduced protein
  - Similarity of the introduced protein to known allergens
  - Susceptibility to enzymatic digestion and/or heat stability

Currently, no single test can predict food allergy for humans (weight-of-the-evidence approach)

- CODEX recommended allergy assessment

  - *If introduced protein from a non-allergenic source*
    - assess amino acid sequence similarity to known allergens
    - assess pepsin resistance

  - *If introduced protein from an allergenic source*
    - assess amino acid sequence similarity to known allergens
    - assess in vitro pepsin resistance
    - assess specific IgE binding
    - assess skin prick testing on appropriate individuals

Short contiguous amino acid matches- window size should be scientifically justified
  - ‘scientifically defensible window size’

- CODEX recommended allergy assessment
  - Other considerations
    - Exposure level of the introduced protein
    - As science and technology evolves other methods may be considered
      - targeted sera screens
      - animal models
      - examination of newly expressed proteins for T-cell epitopes and structural motifs associated with allergens
Weight-of-the-Evidence Approach

- Source of gene(s) / Crop
- Structural features of protein
  - amino acid sequence comparisons
- Biochemical / biophysical characteristics
  - pepsin resistance
  - post-translational effects
- Abundance in crop / food
Consistencies Across Recommendations

- Avoid introduction of known allergens
- Protein from allergenic source or has significant amino acid sequence identity, conduct specific IgE binding studies
- In vitro pepsin resistance
Differences Across Recommendations

- Decision tree vs. weight-of-the-evidence
- 6 vs. 8 or greater contiguous identical amino acids
- In vivo clinical testing
- Inclusion of non-validated methods
  - Animal models
  - Targeted sera screening
    - The value of targeted sera screening, as recommended by FAO/WHO 2001, has not been fully characterized or validated (Thomas et al., 2007, Toxicol. Sci. 97:27-31).
Protein Allergenicity Safety Assessment

- **Current State** - multiple documents/differing recommendations have resulted in confusion and arbitrary inclusion of tests. Decisions based on non-validated (e.g. animal models) or refuted (i.e., 6 amino acid matches) tests.
  - not consistent across geographies
- **FAO/WHO 2001** - continual impact
Desired “Future” State

- Harmonization of testing requirements across geographies (Codex?)
- Inclusion of endpoints based on ‘sound science’ (peer reviewed published data)
- Use of only ‘validated’ endpoints for safety assessment purposes