

Allergens – It's Really Just a Management and Communications Issue

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Protein Trends & Technologies Seminar

Oak Brook, IL, May 5-6, 2015

**Food Allergy
(Food Hypersensitivity)**

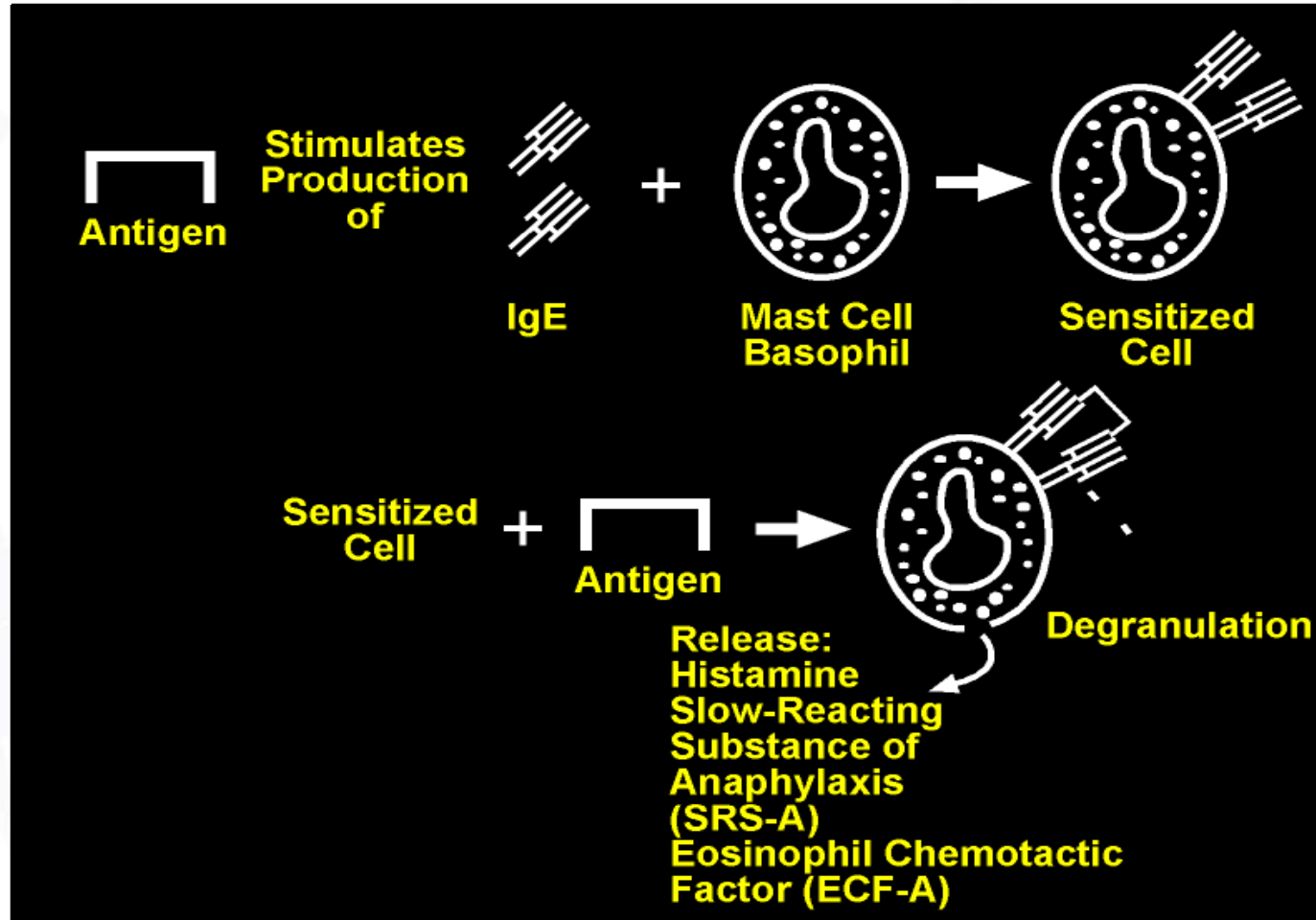
IgE-Mediated

Cell-Mediated

Exercise-induced

Celiac Disease

Mechanisms of Mediator Release



The Big 8

Most Common Causes of Food Allergy (IgE-Mediated)

- Cows' milk
- Egg
- Crustacea
- Fish
- Peanut
- Soybean
- Tree nuts
- Wheat

Responsible for 90% of all food allergies globally

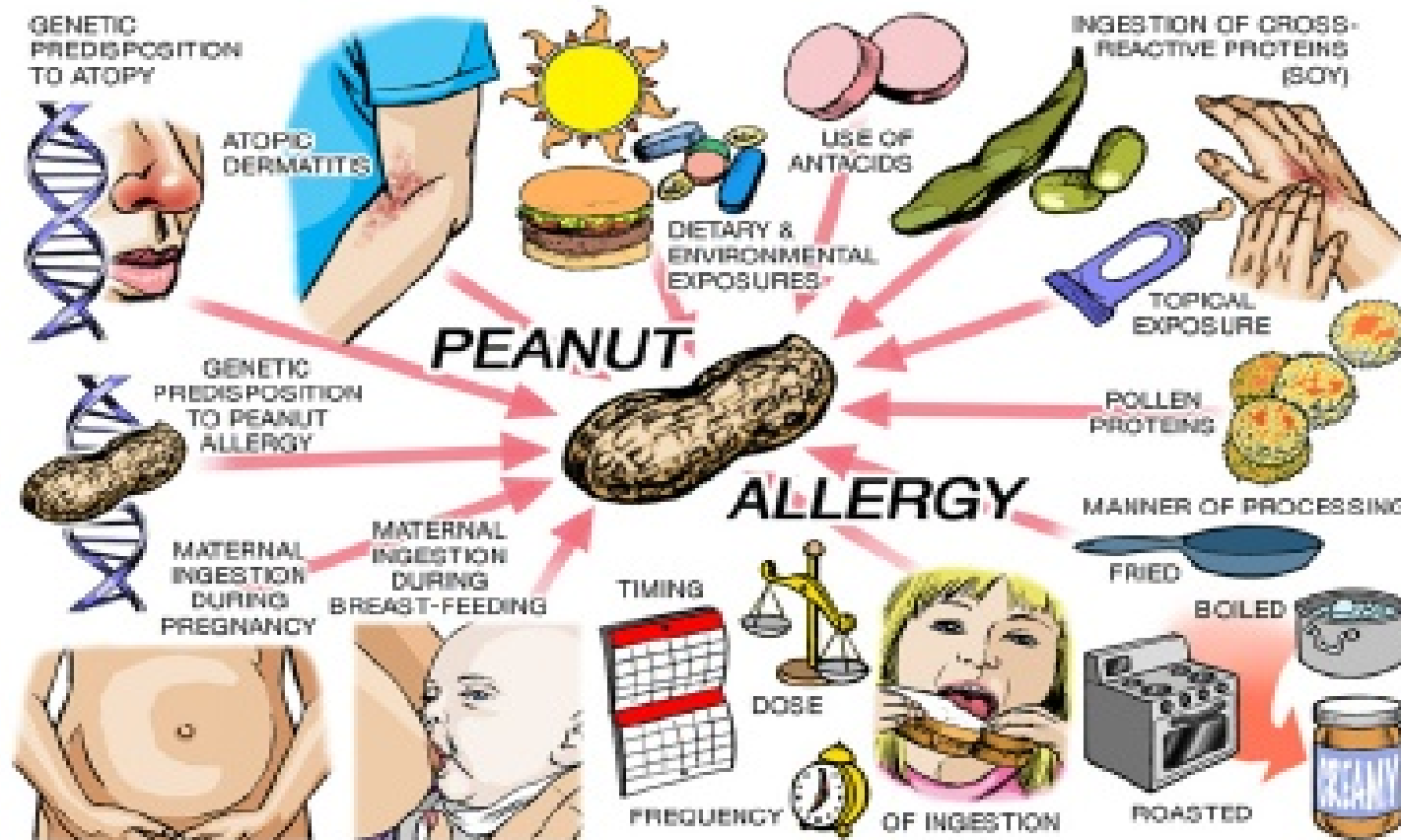
Allergenic Foods on Common Allergen Lists in Other Countries

- Sesame seed Canada, EU, Australia/
New Zealand
- Mustard EU, Canada
- Celery EU
- Buckwheat Japan, Korea
- Molluscan Shellfish Canada, EU
- Lupine EU

Characteristics of Commonly Allergenic Foods

- Frequently consumed
- Consumed in relatively large amounts
- Consumed in early life stages (with exceptions)
- Excellent sources of protein
- Allergenic sensitizing capacity of individual proteins in the food matrix

Factors Involved in Development of IgE-Mediated Food Allergy



Sicherer and Sampson. J Allergy Clin Immunol. 2007; 120:491-503.

Could Novel Food Sources of Protein Become Allergenic?

- Inevitable; reports of allergic reactions should not be a surprise
- But perhaps not commonly; prevalence will be predictable to some extent
- Not all adverse reactions will be allergic reactions (Quorn)
- Should not be a deterrent to development of new protein sources
- Clear labeling is the key

Could Novel Food Sources of Protein Become Allergenic?

- Soybeans
- Kiwi
- Sunflower seeds
- Lupine
- Wheat protein isolate

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Can We Predict the Allergenic Potential of Novel Food Sources of Protein?

- Is the food already known to be allergenic when consumed in more limited quantities?
- Is the food commonly allergenic in another country where it is more commonly consumed?
- Is the food botanically related to known allergenic foods?
- Does the food contain a potentially cross-reactive protein?

Prediction Examples

- Cottonseed protein – severe allergy cases reported from limited consumption
- Canola protein – very closely related to mustard
- Buckwheat – commonly allergenic in Japan and Korea
- Lupine – a legume (but there are 100s)
- Pea – a legume (but there are 100s)
- Insects – potentially cross-reactive protein with crustacean shellfish

Recommended Actions

- Search global clinical allergy literature for evidence of allergic reactions to the novel food
- Identify and assess allergenicity of botanically related species
- Determine abundance and amino acid sequence homology of individual proteins that are from the same class as major allergens from closely related sources
- Test for cross-reactivity and stability

Predicting the Allergenicity of Individual Food Proteins

- Humans consume 1000s of plant and animal proteins in the diet on a daily basis; all dietary proteins are foreign proteins to the human immune system
- Only a small number of proteins from plant and animal origin cause an IgE-mediated immune response in only a small number of humans
- Oral tolerance is the homeostatic condition for dietary proteins
- Why are some proteins allergenic while others are not?

Why Are Some Food Proteins More Allergenic?

- Comparative abundance
- Usually major proteins of the food
- Heat-resistant
- Resistant to digestion/proteolysis
- Resistant to extremes in pH
- Foods can have 1 or many allergens in them

Characteristics of Food Allergens

- Abundance:
 - Most of the major food allergens are present at levels $>1\%$ of the total protein in the food
 - Immune system more likely to encounter abundant proteins
 - Seed storage proteins are especially abundant
- IgE-binding epitopes:
 - Most food allergens have multiple, linear epitopes

Characteristics of Food Allergens

- Resistance to denaturation and digestion:
 - Highly stable proteins have the opportunity to interact with immune system for longer periods
- Allergen structure:
 - Secondary and tertiary structure may contribute to stability
 - Many food allergens contain disulfide bonds that stabilize the protein structure
 - Other allergenic food proteins form aggregates that enhance stability

Classification of Food Allergens

- Food allergens are commonly classified into families by their shared amino acid sequences and conserved 3-D structures
- Bioinformatics analysis indicates that the majority of food allergens from plant sources fall in 4 families
- Food allergens from animal sources fall within 3 main families

Classification of Food Allergens

- 4 main families of plant-based food allergens:
 - Prolamin superfamily
 - Cupin superfamily
 - Bet v 1 family
 - Profilins
- 3 main families of animal-based food allergens
 - Tropomyosins
 - EF-hand proteins
 - Caseins

Prolamin Superfamily

- Characterized on the basis of the presence of a conserved 8 cysteine amino acid residue pattern $CX_nCX_nCCX_nCXCX_nCX_nC$
 - Stabilizes protein structure which contributes to overall allergenicity of proteins in this class (highly resistant to heating, proteolysis and digestion)
- Share a common 3-D structure consisting of bundles of 4 α -helices stabilized by disulfide bonds
- Includes cereal prolamins, 2S albumins, non-specific lipid transfer proteins, and α -amylase and trypsin inhibitor protein families

Prolamin Superfamily

- Gliadin
 - Major seed storage protein from wheat
 - Major component of gluten
 - Involved in gluten sensitivity (celiac disease)
 - Related proteins in barley, rye, triticale, spelt
- nsLTPs
 - Facilitate transfer of phospholipids; plant defense against fungal and bacterial infections
 - Mal d 3 (apple), Pru ar 3 (cherry), Pru p 3 (peach), Jug r 3 (walnut), Cor a 8 (hazelnut), Aspa o 1 (asparagus), Lac s 1 (lettuce), Bra o 1 (cabbage)

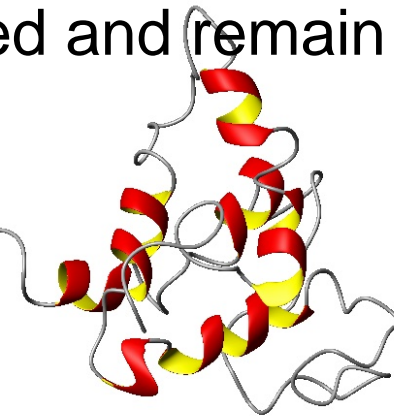
Prolamin Superfamily

- α -amylase/trypsin inhibitors
 - Provide protection against degradative proteases produced by insect pests and pathogens
 - Rag 1, 2, 5 (rice)
- 2S albumins
 - Seed storage proteins
 - Jug r 1 (walnut), Ber e 1 (Brazil nut), Ana o 3 (cashew), Sin a 1 (yellow mustard), Bra j 1 (black mustard), Ara h 2, 6, 7 (peanut), Ses i 2 (sesame), SFA-8 (sunflower)

Prolamin Superfamily

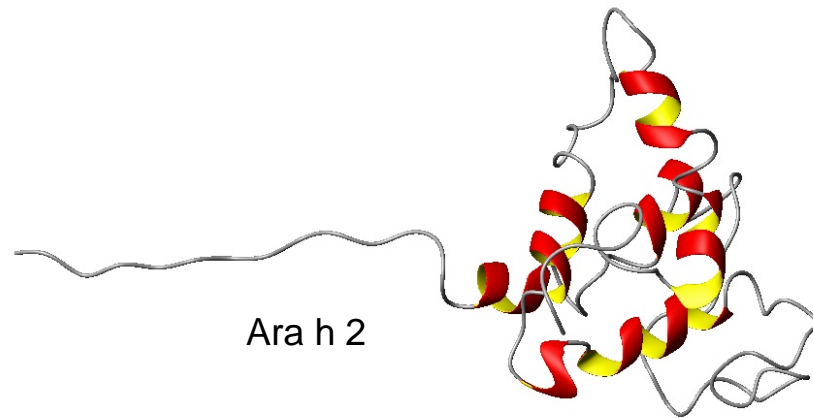
- 2S Albumins:
 - Seed storage proteins of dicotyledonous plants
 - Synthesized as single chains of 10-15 kDa
 - Many post-translationally processed into small and large subunits joined by disulfide bonds
 - Peanut and sunflower are not modified and remain as a single unit
 - Ara h 2 has some trypsin inhibitor properties

Ara h 2



Prolamin Superfamily

- 2S Albumins:
 - Major allergens in peanut, mustard, sesame seed, walnut, cashew, sunflower seed
 - Involved in provocation of severe allergic reactions

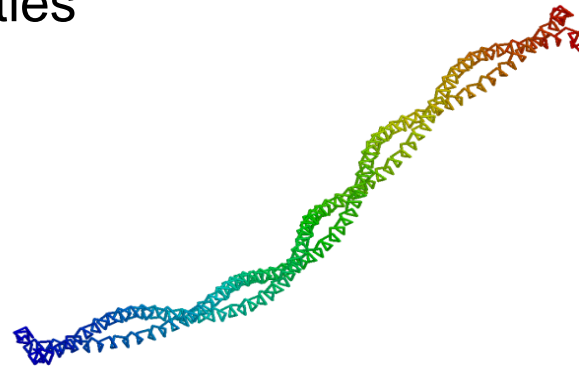


Novel Legume Source Recommended Approach

- Expression level of 2S albumin?? – soy vs. peanut
- Amino acid sequence homology with Ara h 2 from peanut??
- Ability to provoke mediator release from basophils armed with IgE antibodies of peanut-allergic individuals??

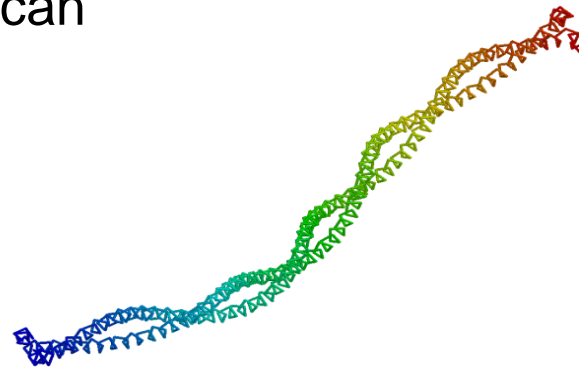
Tropomyosin Superfamily

- Tropomyosins are a family of closely related proteins present in all eukaryotic cells
 - Play a key role in regulation of muscle contraction
- Major allergens in crustacean and molluscan shellfish
 - Pen a 1 (shrimp), Cha f 1 (crab), Tod p 1 (squid), Hal d 1 (abalone), Cra g 1 (oyster), Hel as 1 (snail)
- Inhalant allergen from mites and cockroaches
 - Minor inhalant allergen in most societies



Tropomyosin Superfamily

- Only non-vertebrate tropomyosins have been identified with allergenic activity
 - Differences in the IgE binding epitopes in the C-terminal portion of tropomyosin results in no cross-reactivity to vertebrate tropomyosin
 - Of those that are allergenic, they have been shown to be highly cross-reactive
- Resistant to heat and digestion
 - Tropomyosins are water soluble and can withstand boiling



Novel Insect Source Recommended Approach

- Likely to express invertebrate tropomyosin
- Amino acid sequence homology with shrimp tropomyosin??
- Ability to provoke mediator release from basophils armed with IgE antibodies of shrimp-allergic individuals??

Conclusions & Recommendations

- Novel food sources of protein will inevitably be allergenic
- Thus, be careful about marketing these foods as non-allergenic
- It is more accurate to state that these food sources contain no commonly allergenic food
- But be careful of potential for cross-reactivity with commonly allergenic foods

Conclusions & Recommendations

- Novel food regulations in Canada and EU will require assessment of allergenic potential
- Be cautious in U.S. and other countries where the regulatory path forward on novel foods is unclear; you might simply get blocked
- Example: lupine in the U.S.

Conclusions & Recommendations

- Allergenic potential should not be a deterrent to marketing of novel food protein sources
- Clear labeling is the key
- With clear labeling, those consumers who develop allergic reactions will be able to avoid the food
- Preventive allergen controls will allow management of allergen risks within manufacturing facilities



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