Reconciling Actual Risks of Food Allergy with Scientific Risk Assessment, Regulatory and Public Demands

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Food/Feed Safety Assessment

**RELATIVE SAFETY**

Historically: we learned to eat “safely” through experience but some “safe” foods cause disease in some people:

- **Wheat** must be avoided by those with celiac disease
- Most legumes (beans/peas) must be cooked to inactivate lectins and trypsin inhibitors or diarrhea and malnutrition
- **Allergic individuals** must avoid specific foods causing their allergy

We can markedly reduce big-risks from new sources (Genetically Modified Organisms, novel food Ingredients), but we can not yet guarantee lack of immunogenicity, allergy or absolute safety!
Normal Immune Response to Dietary Proteins

- Tolerance
- Ignorance
- True for most proteins, most foods, most people
- Allergic reactions and celiac, relatively rare compared to influenza...etc.
- Great range of responses to dietary proteins....all allergic responses are not equal, allergens are not all equal
Food Allergy — Growing Concerns / Perceived Risks from different perspectives

• Consumers
  – Apparent increasing prevalence / diagnosis of food allergy
  – Few individuals with severe – life threatening, life-long risks
  – Complex foods, multiple sources
  – Unclear labeling, news reports & social media reports of risks

• Food Producers
  – Liability, regulations, recalls
  – Labeling, sourcing, value added product competition
  – International trade, languages, different regulations

• Regulators
  – Consumer pressure
  – Uncertain scientific methods for risk evaluation
  – Complex detection issues
  – International trade – complexity of regulation and foods
  – Large / small producers, restaurants, packaged foods
Are There Cures for Celiac or Food Allergy?

• Not in the near future.....therefore:
• AVOID YOUR ALLERGEN or Gluten...IF you are sensitive
• Allergenic foods and gluten must be labeled accurately to protect those with allergies or celiac disease!
Types of Foods / Sources and Processes Potentially Requiring Regulatory Assessment: USA, EU, Japan…

• Genetically Modified Organisms (plants, animals, fungi, prokaryotes)
• Cloned animals
• Purified ingredients from allergenic or sometimes highly divergent sources (e.g. any legume)
  – Proteins (or ingredients with potential protein contaminants such as flavor ingredients, gums, new sweeteners, oils, starch, mucins)
• Processed “hypoallergenic” foods

• **Goal:** Protect specifically allergic consumers
• Intent to predict “new allergens”, new sensitization?
• **CAUTION:** Perform “scientifically interesting” tests at your own peril. Those results can impact possible approvals!
GMOs...detailed food allergenicity assessment

- FDA wants early consultation, tests on case-by-case
- EPA more stringent and less communicative
- Many countries want full submission of proscribed studies, no discussion
- EFSA (European Food Safety Authority) keeps pushing toward animal model tests for some GMO, and evaluate potential changes in endogenous allergen expression, transcriptomics etc. They have suggested adding immunogenicity and immunotoxicology.... AND POST-MARKET SURVEILLANCE
Novel Food Ingredients…detailed allergenicity assessment requirements less clear

- FDA wants developers to have early conference to agree to study process on case-by-case: GRAS or New Food Ingredient
- EU and some other countries have specific requirements, but not procedures
- There has been less effort to develop consistent guidelines, primarily focused on avoiding risk of exposure to major allergenic proteins
- PRECAUTIONARY ENVIRONMENT is becoming common
Food Safety Evaluations: We need to differentiate…

- Scientifically justified risks / concerns
- Scientifically justified risk assessment
- Scientifically justified control measures

From

- Basic “interesting” scientific questions without direct relevance to safety (e.g. unintended effects)
- Irrational fears and unsubstantiated speculation
- Non-predictive tests
- Impractical and overly restrictive controls
Immunological Adverse Food Reactions

IgE-Mediated allergy

- Systemic (Anaphylaxis)
- Oral Allergy Syndrome
- Immediate gastrointestinal allergy
- Asthma/rhinitis
- Urticaria
- Morbilliform rashes and flushing
- Contact urticaria

Non-IgE Mediated Cell-Mediated

- Celiac Sprue
  - Protein-Induced Enterocolitis
  - Other Protein-Induced Enteropathy
  - Eosinophilic proctitis
  - Dermatitis herpetiformis
  - Contact dermatitis
Prevalence and Severity

• Celiac Disease affects nearly 1.3% of the world population
  – Genetically linked MHCII DQ2 and DQ8 (but >20% of all people are DQ2 or DQ8, small # have disease)
  – Small percent with celiac must avoid even 100 mg of wheat, barley or rye grain

• Food Allergy 3-6% of world population
  – >20 genes, none are dominant
  – Hundreds of foods, few proteins (out of hundreds) in each food are allergens ALLERGENS are NOT all EQUAL!
  – Few allergenic foods cause life-threatening allergies
  – 0.8% in USA allergic to peanut, 1% to 5% of those have severe allergies and are at risk of systemic anaphylaxis …rice, beef, chicken and bacteria rarely cause food allergy
Celiac Disease with wheat gluten: variable exposure effects

Almost normal intestine, but eosinophils in tips of villi, mild inflammation

Severe celiac inflammation, flattened villi, malabsorption, wasting disease, autoimmune

T-cell and IgG against modified peptides (self-transglutaminase) and natural peptides, and finally modified human connective tissue---autoimmune disease
IgE Mediated Food Allergy Sources
Diagnosis of IgE Mediated Food Allergies

In Vivo
- Clinical history
- Elimination diet
- Skin Prick test (SPT) with extracts or prick-to-prick
- Food challenge
  - Placebo and suspected food

In Vivo (challenge) Limitations
- Some risk to patient
- Time consuming (full-day)
- Qualitative
- Only test one or two foods

In Vitro (IgE)
- Specific IgE measurement
  - CAPS (Pharmacia Diagnostics)
  - RESEARCH METHODS
  - ELISA/RAST
  - Western blots
- Positive result suggest, DO NOT prove allergenicity
What is IgE mediated food allergy – symptoms - pathology

Food allergy causes more than just a runny nose or urticaria!

Sometimes mixed IgE, T-cell and eosinophil reactions
During a food challenge. Some risk

The unexpected… 15 minutes

But she did recover: Epinephrine, IV Other support Clear anaphylaxis

Could have died if this was not in the clinic
Food Allergy Prevalence
(apparently increasing estimates from US population of 300 million)

- ~ 30% of people have allergies to inhaled allergens
- IgE mediated allergies (Type I) is the most common type
- Occurrence of food allergy in the US and Europe
  - 2-4% of adults
  - 4-8% of young children
  - Severe reactions relatively rare (U.S. estimates: ~100,000 Emergency Room visits, < 200 fatal reactions /year)

- Eight foods account for ~ 90% of food allergies & even minor ingredients require labels (US), 14 EU...some countries do not:

  Peanuts  Milk  (Wheat?)
  Eggs     Fish  (Soybeans?)
  Crustacea Tree nuts

The EU adds lupine, celery root; mustard and sesame seeds
India should add chickpea, blackgram, lentil, pigeon pea?
Known Allergenic Proteins
Very few foods or proteins represent major risks

• Peanuts
  – Probably ~ 50 to 80+ deaths per year in the U.S.
  – 3 to 5 major allergens, 5 to 7 minor allergens
  – 10,000-40,000 total genes

• Soybeans
  – < 1 fatal reaction per year in the U.S.
  – 3 to 5 moderate allergens
  – ~20,000 total genes

• Cow’s milk
  – Few published reports of fatal reactions (e.g. Macdougall, 2002)
  – Caseins and beta-lactoglobulin dominant allergens, also alpha lactalbumin, minor allergens IgG, serum albumin

• Fish
  – Few reports of fatal reactions, but strong reactions common
  – 1 major allergen (parvalbumin), 2 to 4 minor allergens
IgE – allergy: Sensitization vs. Tolerance

**Allergy**
- Mast cell
- IgE-receptor
- Histamine

**Mast cell**
- Protein digestion
- Antigen (Ag) absorption
- Ag processing in DC, Mφ, B cells
- Ag presentation to T cells
- T cell and B cell memory

**IgE-receptor**
- IFN-γ
- TNF-α
- IL-5

**B cell**
- IL-4
- IL-13

**T h cell**
- IL-2
- TGFβ

**T reg cell**
- IL-4
- IL-13

**Non-IgE-Mediated**

**Ig**
- IgG
- IgA

**EOS**
IgE Mediated Symptoms 10 to 20 minutes after eating:
- hives
- angioedema
- asthma
- diarrhea/vomiting
- atopic dermatitis
- anaphylaxis

**Elicitation:** Protein-specific IgE is the key mediator of specificity in Food Allergy

**Sensitization**

- Antigen Specific
- B cells Make IgE

Peanuts (Ara h 1)

- (2 IgE epitopes)

**IgE**

**FcεRI**

Mast cells release histamine & leukotrienes
Potential IgE Antibody Binding Epitopes:
Peptides - amino acids fixed in spatial arrangement – rarely to N-linked carbohydrate

Conformational or discontinuous IgE epitope
Often heat labile

Sequential or Linear IgE
Usually heat stable

A Few Specific Asparagine-linked Glycans bind IgE but symptoms & disease is rare
Assessing the Potential Allergenicity of GMOs and Novel Food Ingredients (in order of risk)

1. Does the gene encode a protein that is known to be an allergen (or celiac)? (Evaluate source… and the protein sequence… bioinformatics, similar to an allergen?), serum IgE tests if necessary

2. Is the protein sufficiently similar to an allergen (or celiac inducing protein) to expect cross-reactions? (bioinformatics), serum IgE tests if necessary

3. Is the protein likely to sensitize and become an allergen? (e.g. stable in pepsin, abundant and stable to heating)
Interpretation of Codex: Goodman et al., Nature Biotech Jan. 2008
Assessing the Potential Allergenicity of GM Crops – What Makes Sense?

Weighing results from tests with imperfect correlations (Codex 2003)

- **Source of gene**
  - No reports of allergy
  - Allergy common

- **Protein sequence compared to known allergens**
  - < 35% identity over 80 aa
  - > 70% identity over full length

- **Reliable prevalence data**
  - Proof of allergic responses

- **Quality of database**
  - Proof of allergenicity

- **Additional factors:**
  - Abundance in food
  - Stability in heat/processing

- **Pepsin digestion**
  - Digested > 90% in < 2 mins.
  - Stable 60 mins.

- **Perform serum IgE tests:**
  - IF source is allergenic
  - IF protein match > 35%

- **Serum donors:**
  - Clinical proof
  - Tests: specific inhibition

Accept | Label or Reject

Specific IgE = to allergen
Sequence comparisons may predict cross-reactivity

1. Overall FASTA in AllergenOnline (>50% identity = structural similarity and modest to significant chance of cross-reactivity)

2. FASTA scanning 80 aa window (79 aa overlap), (>35% identity = some possibility of cross-reactivity)

3. Scanning 8 amino acid identity NO PROVEN VALIDITY

Allergenonline Homepage ver11
http://www.allergenonline.org
Can we predict which proteins are going to be allergens?

• Predicting B cell epitopes is imperfect
  – IgE and IgG epitopes often share the same epitopes
  – Very individualistic based on limited data

• T cell epitopes more straight-forward? but which are Th1, Th2, Th3? Or cytotoxic? And can the same epitope have multiple functions
Mapping IgE epitopes: Imperfect and Limited Data (few allergens, few patients)

Bovine beta lactoglobulin, IgE epitopes
Jarvinen … Sampson, 2001 IAAI 126:111

11 subjects over 3 year old: pooled

8 subjects under 1 year old: pooled

**Fig. 4.** Cumulative OD scores of IgE antibodies for each of the synthetic decapeptides of BLG. According to the known AA sequence, 77 decapeptides, overlapping by 8

**Fig. 5.** Amino acid sequence of BLG. IgE binding epitopes are shown in bold. IgG binding epitopes are underlined.
Potential cross-reactivity CAN NOT be predicted by 3-D comparison w/o IgE.

Allergen          % Identity to Ara h 1

- Peanut Ara h 1    100%
- Hazelnut Cor a 11  34%
- Walnut Jug r 2    35%
- Soybean β-CG      51%

3D structures based on soybean β-CG

There is very weak IgE cross-reactivity from peanut to tree nuts.

Magenta = carbohydrate, other colors represent putative shared IgE epitopes.

Barre et al., Mol Immunology 2008, 45:1231-1240
Peanut Ara h 1 Search AllergenOnline deciding which proteins to test!

Table 1a) Sequence matches to peanut Ara h 1 GI:1168390

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Potential IgE binding to Asparagine-Linked Glycans (~1200 structures– Some bind IgE of some allergic subjects…are they allergenic?

**Fig. 3** Structures of Representative N-glycans of Glycoproteins used in the Study Containing Antigenic CCD Epitopes

*Can bind IgE*

- Insect CCD can bind IgE
- Animal glycoproteins – usually no IgE binding…

Goodman
Animal glycan exception: Galactose $\alpha 1-3$ Galactose

Non-primate Alpha-gal IgE binding & allergy associated with tick bites
Commins & Platts-Mills, 2009

- IgE immediate reactions to i.v. monoclonal antibody produced in CHO cells...in some tick bitten patients
- Delayed anaphylaxis to beef, pork for similarly IgE sensitized patients
- IgG responses from xenographs of pig tissue...extra-cellular matrix glycoproteins...rejection
Serum IgE tests: must be reliable, sensitive and specific

The ideal serological IgE immunoassay

- True Clinically Allergic Subjects
- True Non-Allergic Subjects

Specific IgE Quantity

# of People

Cut-off

Not allergic

allergic
Serum IgE Tests – based on source of gene or sequence match

- Must be specific
- Require validation
- Positive and negative control allergic sera
- Positive and negative control allergenic proteins & extracts

See study designed by Goodman & Vieths:
Hoff et al., 2006. Serum testing GM soy. Mol Nutr Food Res 51: 946-955
Antibody tests are needed sometimes as computer predictions do not PROVE allergy

- **Dot blot** ~ microarray
- **Immunoblot**
  - Reducing
  - Non-reducing
  - Native
  - 2-Dimensional
- **ELISA**
- **RAST**
- **EAST**
- **Inhibition in all formats**
  - Protein
  - CCD

![Diagram showing Direct Binding and Inhibition](image)

- **Direct Binding**
  - IgG – anti-IgE with label
- **Inhibition**
  - IgG – anti-IgE with label
  - Soluble Ag inhibitor
Direct IgE Western blot apparent co-sensitization or cross-reactivity? Extracts of legumes (pulses)

Glycoproteins in Navy bean bind IgE from some legume allergic subjects, but it is unlikely to cause allergic reactions.

Fig. 1 Western Blot Under Reducing Conditions

Protein & glycan specific

Protein specific

Soybean Allergic
Soybean & peanut Allergic
Peanut Allergic

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Protein-specific IgE is the key mediator in Food Allergy

IgE binding to one epitope does NOT release histamine or cause symptoms

IgE binding to Cross-reactive carbohydrate determinants...does not (usually) cause histamine release
Histamine release assay from stripped human basophils passively sensitized with highly peanut allergic sera #728

PN = peanut….more than 100 fold stronger
NB = Navy bean
NTP = non-transgenic pea
TP = transgenic pea (aAI)
AlgE = anti-IgE control

Peanut CAPS 76 kU/L
Bean CAPS < 1 kU/L

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Human Lactoferrin vs rhLf expressed in bovine milk Glycan structures

Yu T....Li N, 2011 Glycobiology 21(2):206-224

NO Gal alpha (1-3) gal

Therefore no demonstrable risk

Goodman FARRP
2D-PAGE and IgE immunoblots 4 soybean varieties

Serum # 714
If there was no history of allergy and the sequence was not similar to a known allergen…so no serum testing (no identifiable at risk population)

Is the new protein likely to become a major food allergen?

• Hard to answer with great certainty

• But importantly, low risk….compared to:
  – Transfer of a protein that is an allergen
  – Transfer of a protein that is highly identical to an allergen and likely cross-reactive
Stability of the protein to digestion by Pepsin

- Assay conditions tested in a ring study
- Update: Objective measurements
  - Ofori-Anti et al., Reg Tox Pharmacol (2008)

Provides a correlation for major food allergens.

This test is not meant to “mimic” real digestion.
So far NO non-human animal model has proven predictive of human allergy (for more than a few proteins)

Therefore animal models are NOT useful!
Can Human Cell Based Assays Predict Allergy?

• Probably not at this time
• Few tests with sufficient in vivo human responses to be able to judge the cell assay results: Mixed, some success and some BIG failures
• Need validation with common strong allergens, weak and “non” allergens….or allergenic foods
Working Through Regulatory Hurdles and Food Safety Issues…No Food is 100% safe…

Sometimes the regulatory door seems shut or the walls are high…

…Politics, Economics, Philosophy…

Or scientists seeking absolute answers…

Fight for products that show benefits and have a reasonable safety profile!