Science For A Better Life

How close are we to predicting allergenicity of new proteins?

13 April 2012, Praha

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Agenda/
Content

- Potential risk and basic principles
- Current status in developing a model of human allergy in animals
- Variables associated with development of models
- Challenges in identifying a reliable model
- Standardization / validation
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Categories of Potential Risks

Allergenicity of the protein:

- Transfer a known allergen or cross-reactive proteins → Bioinformatics & immunomethods
- **Creation of *de novo* allergens** → Physical properties of protein

Animal model

Allergenicity of the crop:

- Increase of endogenous allergens in crops → Immunological methods, proteomics

Ideal Animal Model

- Sensitized / challenged via the oral route
- Avoid adjuvants
- Tolerates most food proteins, especially non-allergens
- Provides a relatively easy and reproducible test
- Develops allergen-specific IgE in response to known human food allergens (vs non-allergens)
- Selectively produces a significant amount of IgE
- Relevant to human disease

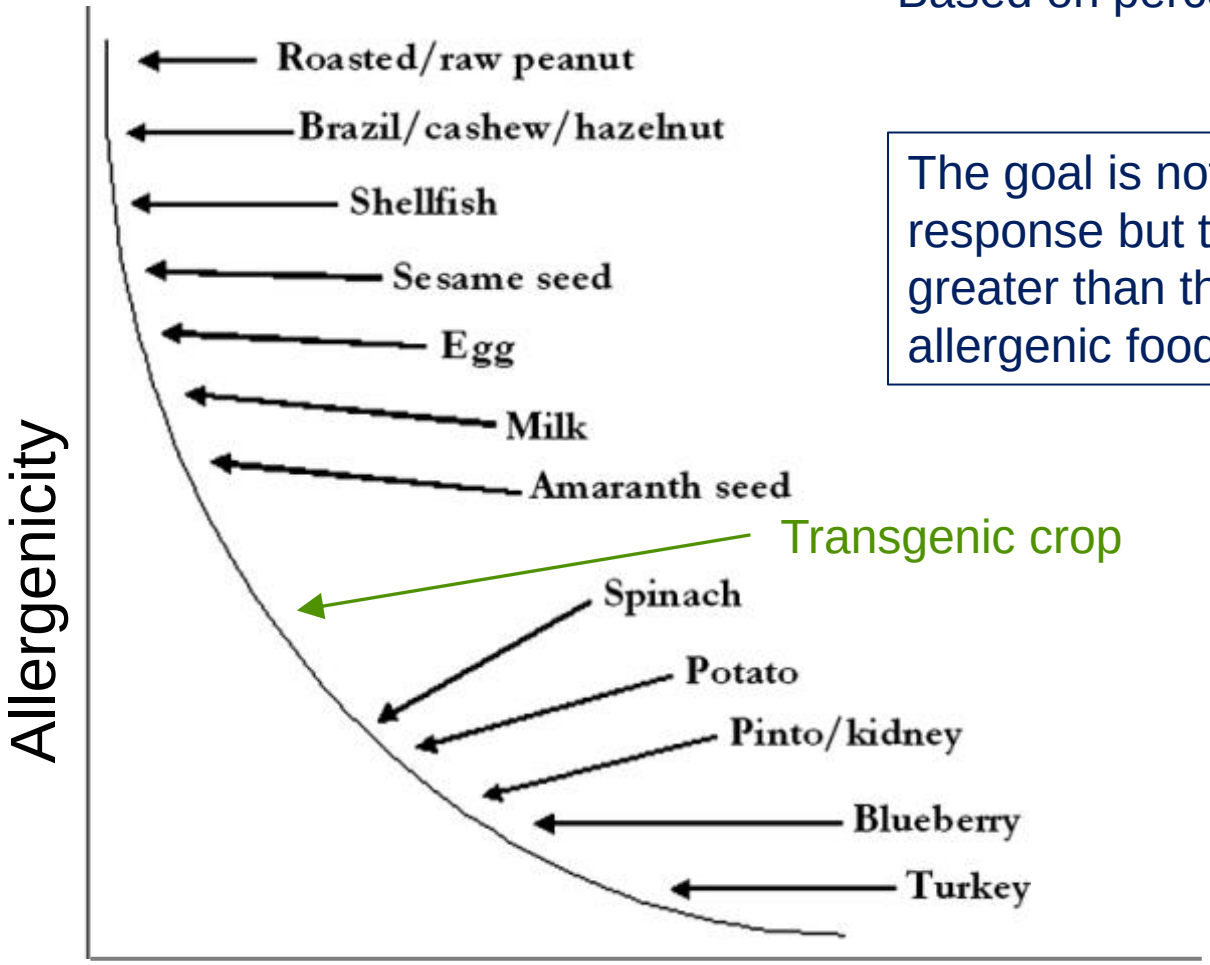
Taylor 2002

Goal : To establish a spectrum of food allergy potencies and then determine where in that spectrum novel proteins or transgenic foods fit.

Spectrum of Allergenicity Potency

Based on perceived allergenicity in humans (Kimber, unpublished)

The goal is not to show an absence of IgE response but to show an IgE response no greater than that associated with most non-allergenic foods and/or proteins.



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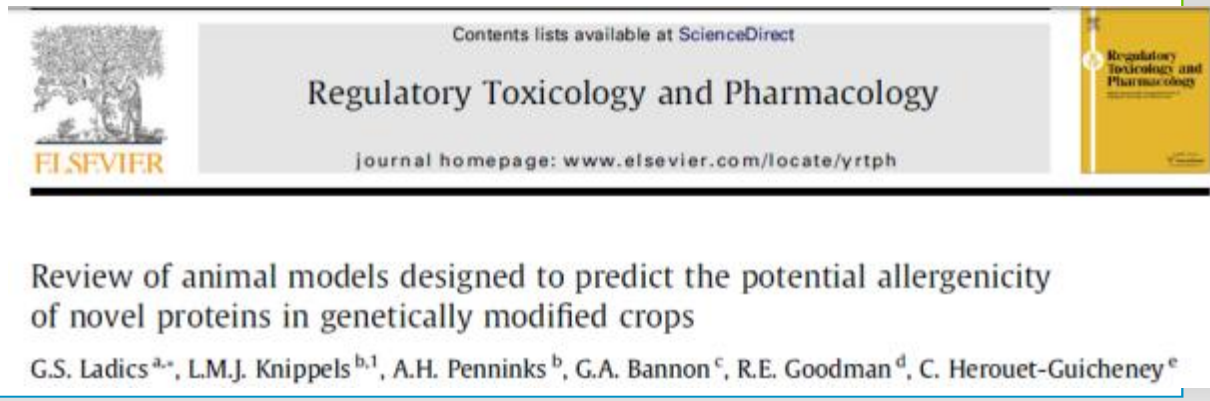
Variables associated with development of a rodent model

Current Large Animal Models

- **Neonatal pigs**:
  - Can quantify physiological parameters of anaphylaxis and skin inflammation that are similar to the human conditions
  - Explore intestinal parameters of allergenic inflammation

- **Dogs**:
  - Bred to be hypersensitive to protein antigens
  - Have shown human-like allergy response to a variety of food allergens

– Parenteral immunization with adjuvant
– Expensive and laborious
– Ethical considerations



Current Rodent Models

- Extensive mechanistic knowledge
- Best opportunity for routine use in allergenicity testing (cost effective, easier to handle)
- Brown Norway Rat (Knipples et al. 1999, Madsen's model) or wide selection of mouse strains (BALB/c , C3H mice, etc)

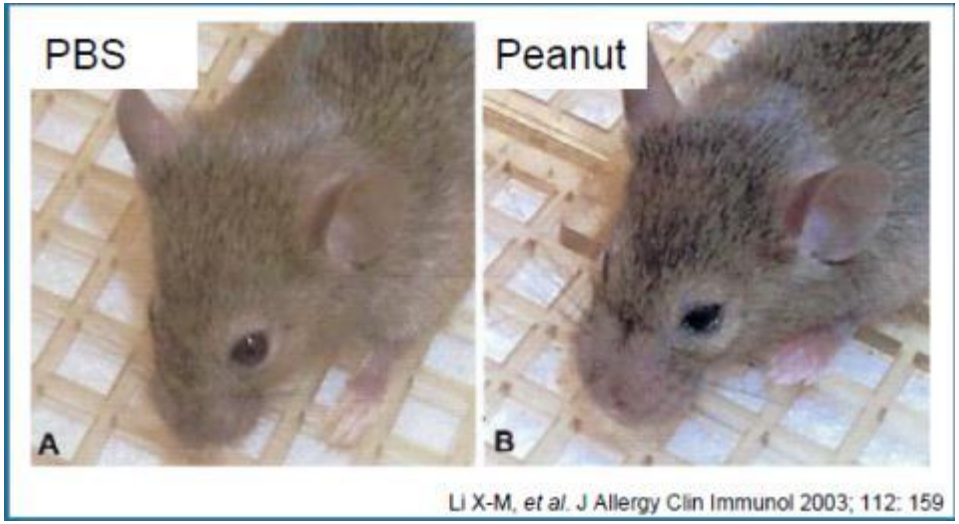
- <u>Sensitization routes:</u>
  - Intraperitoneal without adjuvant (Dearman et al. 2003; Herouet-Guicheney et al., 2009)
  - Parenteral with aluminium adjuvants (Ladics et al. 2010)
  - Transdermal immunization (Lack et al. 2003)
  - Oral
    - Cholera toxin (Li et al. 1999; Bowman & Selgrade 2008)
    - Staphylococcal enterotoxin B (Ganeshan et al. 2009)
    - High dose with antacids
    - Prolonged feeding (daily for 9 weeks)

Animal Strain Differences

Animal genetics can modulate the immunological response

C3H mice that are resistant to endotoxin (LPS) exposure respond differently immunologically than Balb/c.

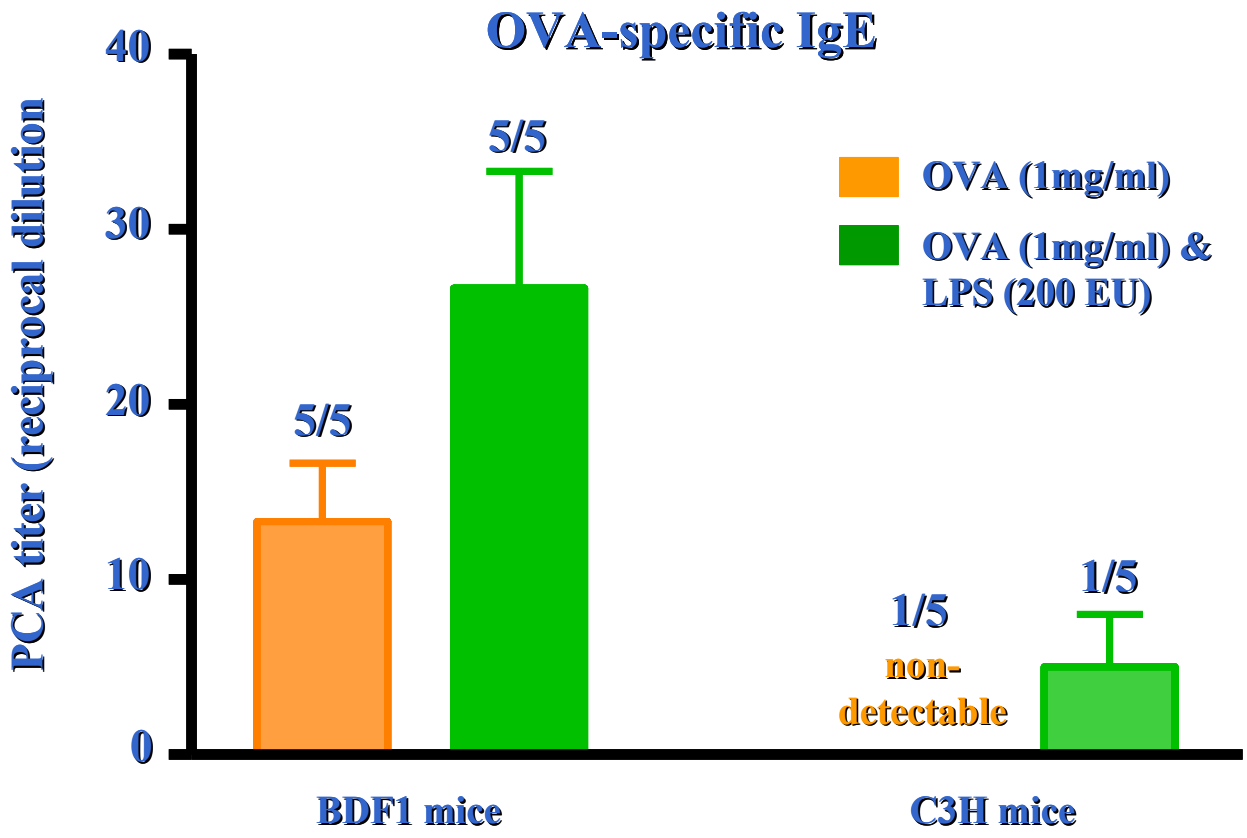
C3H/HeJ mice will develop an IgE-mediated hypersensitivity to human allergens (e.g. peanut and hazelnut), but not to human non-allergens (e.g. potato and spinach)



Oral gavage with peanut and cholera toxin

Animal Strain Differences

Animal genetics in combination with adjuvants can modulate the immunological response



Allergy in Animals versus Allergy in Humans

Fundamental differences with regard to allergenicity in humans versus animal models :

- Rodent models represent in-bred strains
  - Strain selection can be very important in determining the measured response to allergen challenge.

- Humans are an “out-bred” condition:
  - Individual variability in response is unpredictable.
  - Extrinsic factors such as genetic predisposition, disease, age, dietary habits, geographical location, etc are important modulation factors.
  - There is no control over the relative exposure history of the human population for which the prediction is being applied

Characteristics of a Quantitative Model

- Known allergens are currently identified by taxonomic relatedness, clinical prevalence and severity in human population
  - Use of known allergens for establishing a positive response
  - Establish negative controls = non-allergens that produce limited or no response in the model
- Parameters for measuring the allergic response are associated with clinically and/or biologically relevant allergy in humans
- Assay can distinguish human allergens from non-allergens, i.e. to distinguish a threshold beyond which significant allergy would be predicted
- Reproducible

Challenges in identifying a reliable model

Challenges for Animal Model Development

- How to measure the allergenic response in animals?
- How to overcome the oral tolerance?
- Mixtures of allergens versus single pure allergens?
- Route of exposure: for food allergens is it appropriate to orally or systemically sensitize?
- Doses of exposure?
- Effect of food matrix and/or food processing?
- Etc

Selecting Appropriate Endpoints of the Allergenic Response

- Measure physiological parameters that are common among the model and humans
- IgE – an absolute hallmark of allergic potential ?
- Cytokines does not necessarily correlate with IgE levels
- Other biomarkers of allergy/immunological response. What level of the biomarker response is equivalent to a clinically significant response in the animal ?
- IgG – same than cytokines: no determinative (Birmingham et al. 2002)

Measuring IgE: PCA vs ELISA

Determining sensitivity for IgE quantification

Knipples et al. 1998. Clin Exp Allergy.

- ELISA is more sensitive
- When the ELISA was negative so was the PCA
- Western also more sensitive (Knipples et al. 2000)

Reciprocal Titre

IgE (ELISA)	PCA*
9	2
11	3
11	4
5	1
12	-
13	6
12	4
-	-

* Passive Cutaneous Anaphylaxis

Oral Tolerance = Major Barrier to Developing such a Model

- Rodents are prone towards oral tolerance to food allergens. => Difficult to perform oral sensitization schedules

Barone et al., 1998. J Immunol 161:154-160

Atkinson and Miller. 1994. Toxicology. 91: 281-288

Sun et al.,1999. J Immunol 162:5868-5875

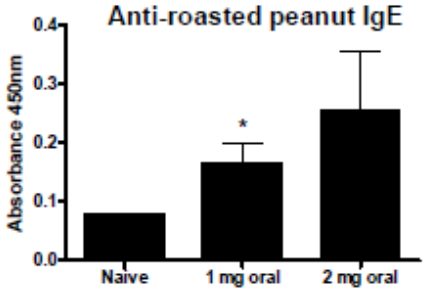
- In rodents, as in most humans, the immune response to an ingested protein (that survives digestion in the stomach) is an active process (oral tolerance) that blocks the development of IgE and delayed-type hypersensitivity responses

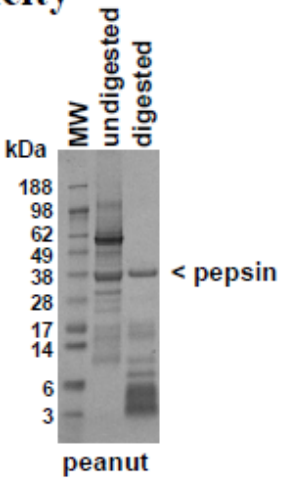
Strobel & Mowat 2006

Pepsin Resistance may be Indicative of Allergenicity

Pepsin stability is clearly important for sensitization but resistance to both pepsin and trypsin appears to be required for oral tolerance

Digestive stability and allergenicity

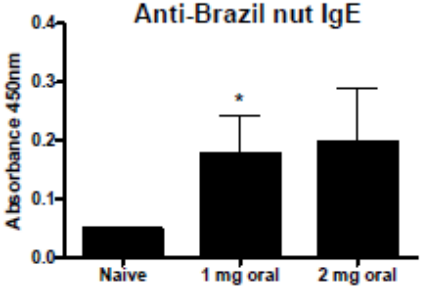




Extracts digested in simulated gastric fluid pH 2.0 for 15 minutes

- Oral exposure w/CT model reflects known allergenicity

Supports current use of pepsin resistance in decision-making process



Bowman and Selgrade 2008

Allergens: Whole Extract or Purified Protein ?

- Form of the allergens/ immunogens used for sensitizing animals can be source of variability in the allergenic response

  Hartl et al., 1999 Int Arch Allergy Immunol

  Lowenstein & Larsen. 2001. Curr Allergy Asthma Reports

  Suck et al. 2002 Allergy

- There is a challenge in selecting a form of the test materials that both primes an appropriate allergenic response AND has relevance to human allergen exposure

  Birgit et al. 2002 FASEB J

- Purified peanut allergens possess little intrinsic immune-stimulating capacity in contrast to a whole peanut extract

  van Wijk et al. 2005 Toxicol Sci

Food Matrix and Food Processing Effects

- Altering stability, pH, and/or solubility of the sensitizing food extract or protein can change the results obtained in the oral animal models

=> Food matrix in which proteins are presented and food processing can affect results

Foss et al. 2006 Int. Arch. Allergy Immunol.
Thomas et al. 2007 Food Chem. Toxicol.
Mills and Mackie 2008 Curr. Opin. Allergy Clin. Immunol.
Dearman and Kimber 2007 Clin. Exp. Allergy
Wang et al. 2009 Plos One
Alvarez and Boye, 2012 J. Allergy

Route of Exposure

Many models, especially rodents, utilize intra-peritoneal or systemic route

- Intra-peritoneal or systemic injections: more control/direct
- Oral: may approximate the digestive route of exposure in humans
- Intranasal: appropriate for some allergens. Food allergen?
- etc

Ladics et al. 2010. Reg. Toxicol. Pharmacol.

Identifying the Appropriate Dose is Critical

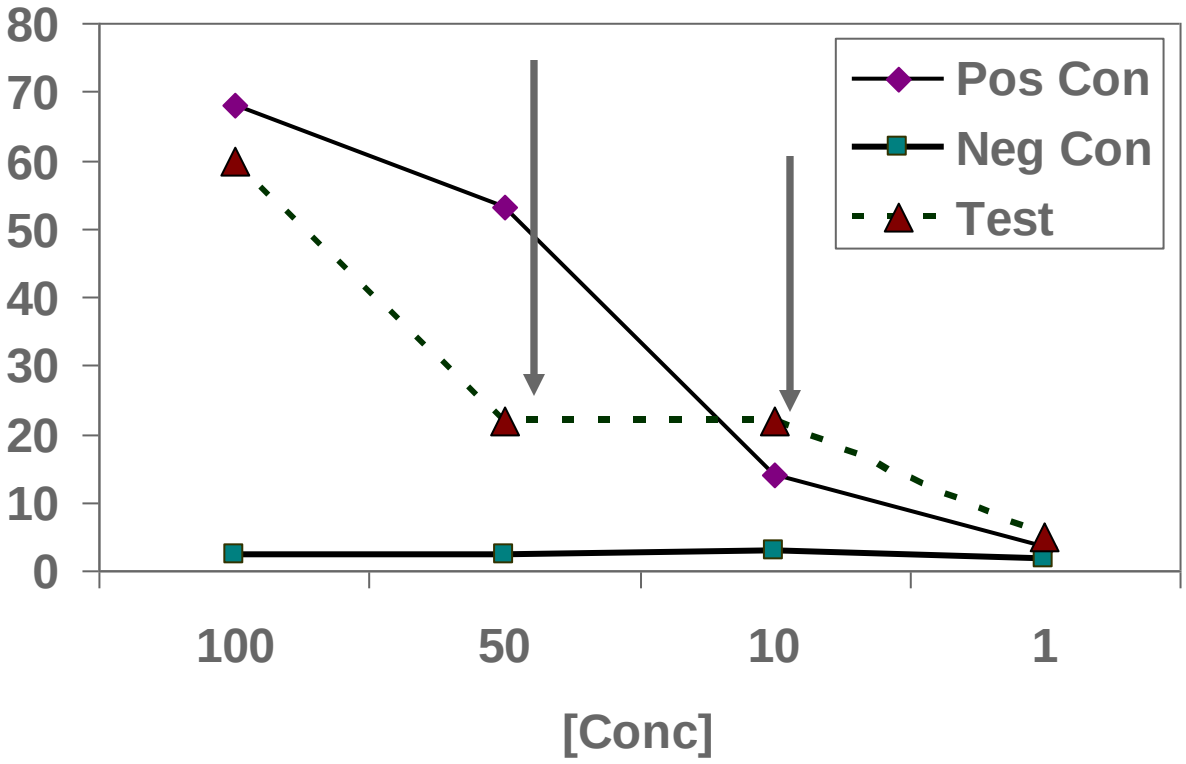
Dose Effects on PCA Results in BDF1 Mice

An increasing dose does not always produce an increased response

	OVA % Dose		b-LG % Dose		CPI % dose	
	.01	0.1	.01	0.1	.01	**0.1**
Day 14	80	15	2	2	*32*	*5*
Day 21	100	200	25	50	*32*	*16*

Identifying the Response Curve

Dose response curve instead of one test concentration: Look for parallelism.
Non-parallel response can indicate dissimilar biological response.



Standardization

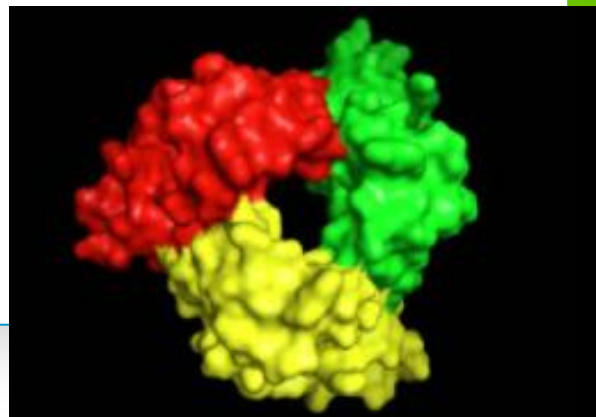
Validation

Standardizing Allergens

- Allergen plant extracts are very difficult to standardize
- It would be desirable to test purified, bacterial-expressed, transgenic/novel proteins. However, this may
  - Not adequately mimic real world exposure
  - Laborious – especially for intractable proteins (e.g. different folding after purification). Positive and negative controls extracts are more easily obtained than purified proteins.
- Solubility: many allergens form multimers and aggregates

  Example: roasted peanut is not very

  Soluble & forms aggregate trimers

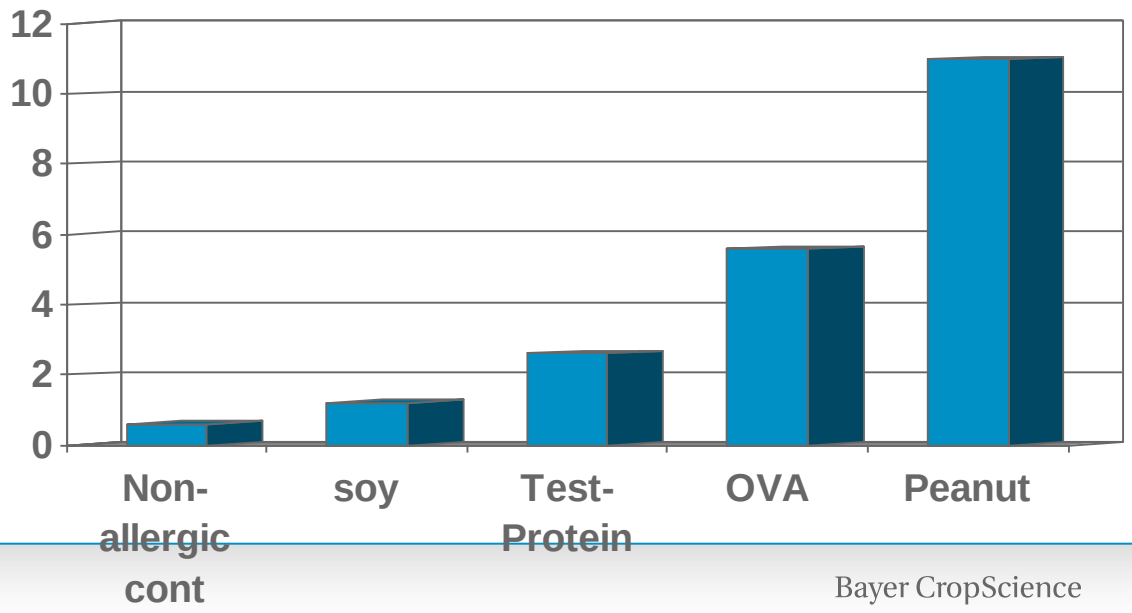


Validation of Model

- For an animal's ability to reliably estimate allergenic potential the assay must be validated for the following criteria:
  - Establish positive and negative controls
  - Report Limits of Detection and Quantitation
  - Accuracy: can the animal reliably produce a scaled response for controls and positive test allergens?
  - Robustness
  - Precision:

Are responses

significantly separated?



Validation Parameters

Reproducibility

- Animal to animal variability
- Test facility variation
- Animal diet variation: tolerance issues
- Antigen/novel protein preparation variability
- Antigen purity/matrix considerations

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Do Animal Models Have Potential to Contribute to Allergenicity Assessment?

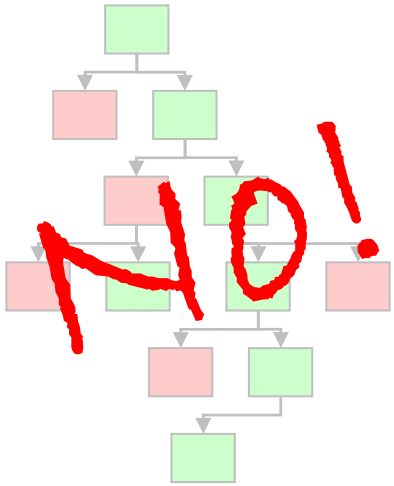
- Animal models are very complex systems that are difficult to understand in the context of human allergy
- Significant progress has been made toward developing animal models; however **none of the animal models have been fully validated as predictive tools of allergenicity potential of food proteins**
- Work remains in establishing validation data for model(s): strains, adjuvants, range of allergens, controls that represent a range from highly (commonly) allergenic to poorly (rarely), allergen extraction, appropriate endpoints, etc.
- Validation would require a coordinated effort among multiple laboratories

Next steps

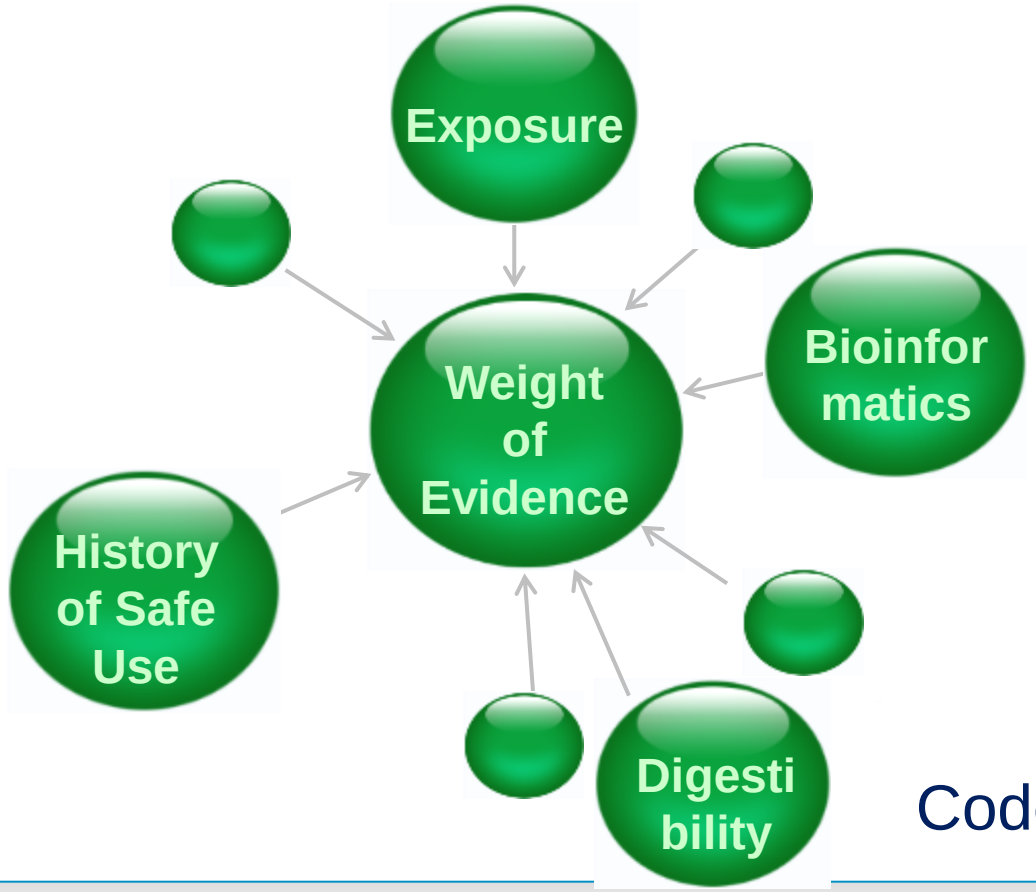
- To further continue assessing the mechanisms of protein food allergy and also tolerance (e.g. relationship between IgE levels and some of the manifestations of disease, an ongoing issue for all atopic diseases)
- To establish threshold levels of sensitizing antigen and challenging antigen doses. Also to resolve the role that matrix plays before designing a validation study….Food extracts versus purified allergens.
- To develop methodologies that will allow standardized methods for monitoring physiological responses in protein food allergy. Additional foods/proteins need to be examined in both models and by other laboratories for validation
- To evaluate their effectiveness and utility in the predictive evaluation of sensitization potential of proteins. One model may not fit all.
- Then to integrate the assay(s) in the Weight of evidence approach.

Current Assessment Approach

- Uses a set of well understood **in silico and in vitro methods** to identify significant characteristics that are common to allergens

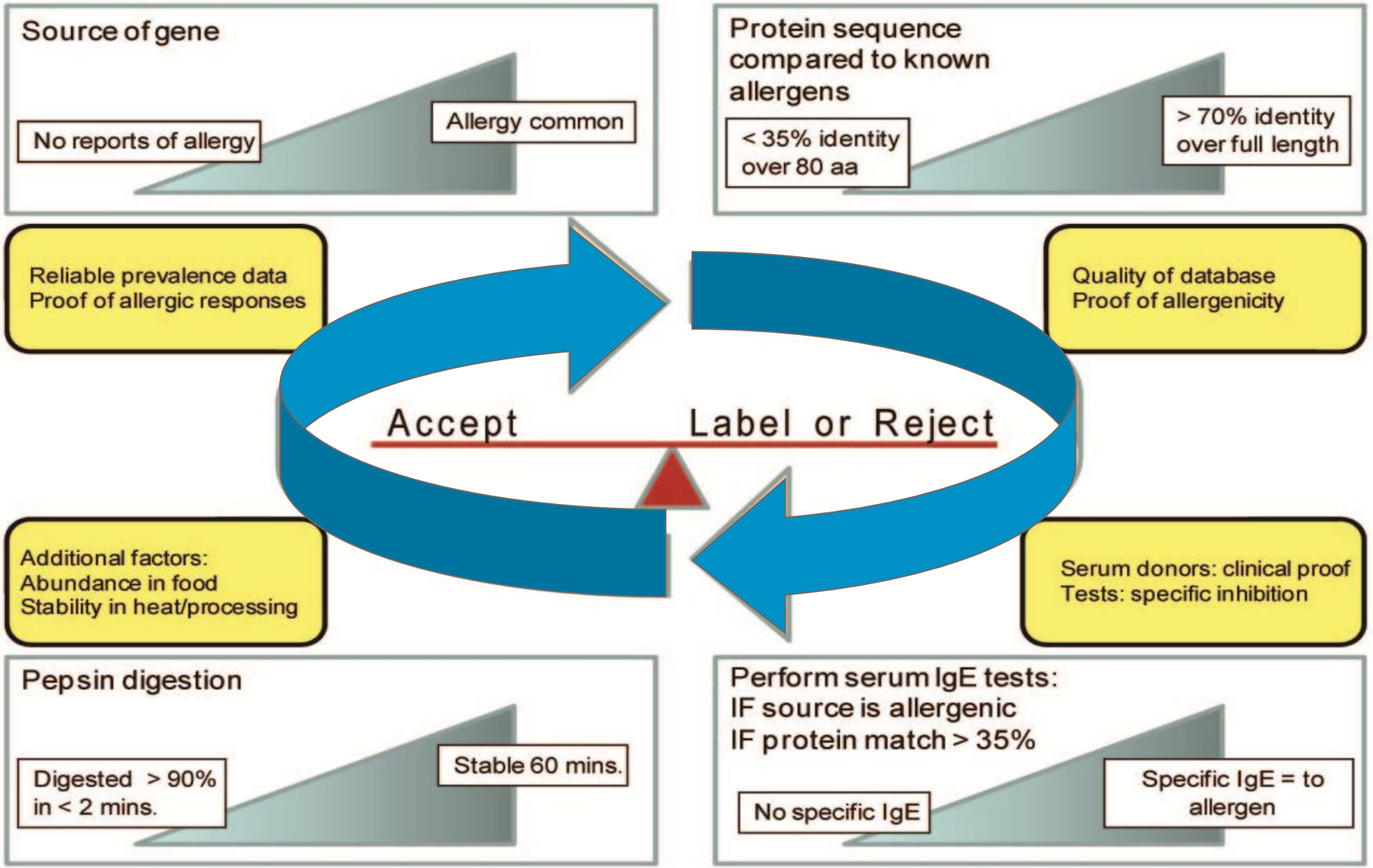


Decision tree



Codex 2009

Weighing results from tests with imperfect correlations



Ideal proteins

- Ideally, the protein(s) should be:
  - From non-allergenic source(s)
  - Sufficiently characterized (familiarity)
  - Not structurally related to known allergens
  - Labile in pepsin stability test
  - Likely to be destroyed/removed after food processing
  - Present at low levels in plants
  - (Not recognized by specific IgE)

Thank you