

UTILITY OF ANIMAL MODELS FOR PREDICTING HUMAN ALLERGENICITY

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OBJECTIVES

- Development of animal model to assess allergenic potential of novel proteins.
- Criteria required for selection, standardization and validation of such a model.
- Facilitate expectations in meeting the potential for predictive testing of novel proteins.

NOVEL PROTEINS

- PROTEINS EXPRESSED IN GM FOODS TO WHICH THERE HAS BEEN MINIMAL OR NO PRIOR HUMAN EXPOSURE.
- THERE ARE SAFETY CONCERNS FOR PROPER RISK ASSESSMENT.
- IS THE STANDARD ALLERGY ASSESSMENT USED FOR THESE MOLECULES SUFFICIENT?
- INTEREST IN ANIMAL MODELS OF ALLERGY FOR TESTING SUCH MOLECULES.

CURRENT NOVEL PROTEIN ALLERGENICITY ASSESSMENT

- BIOINFORMATICS-SEQUENCE HOMOLOGY ANALYSIS USING ALLERGEN DATABASES
- COMPARE TEST PROTEIN WITH KNOWN PROPERTIES OF FOOD ALLERGENS
- TEST SERA OF FOOD ALLERGIC SUBJECTS FOR IGE ANTIBODY BINDING WHEN APPROPRIATE
- 3D MODELING BASED ON ALLERGENIC PROTEIN FAMILIES

FURTHER ASSESSMENT OF NOVEL PROTEINS

- Questions have risen with regard to a need for further allergy safety testing of molecules with no prior human exposure nor significant similarity with allergens.
- Any direct approach of human exposure testing has unacceptable ethical constraints.
- Animal models: potential in vivo allergy exposure testing of food proteins.

ANIMAL MODELS

- MECHANISMS FOR IgE-MEDIATED ALLERGIC RESPONSES.
- THE AFFECT OF PROPHYLACTIC AND THERAPEUTIC INTERVENTION ON FOOD ALLERGY OUTCOME.
- ASSESS PROTEIN ALLERGENICITY.

Choice of Animal Model for Study Of Food Allergy

Approach-Objective	Species	Reagents Available	Size	Relative Cost	Natural food allergy	Adjuvant Needed for Sensitization
Mechanisms	Canine	Some	Lg	+++	+++	
	Swine	Some	Lg	+++		
Improve Therapy	Rodent	Many	Sm	+	-	+++
	Canine	Some	Lg	+++	+++	
	G.Pigs	Some	Lg	+++		
Assess Allergenicity	Rodents G.Pigs	Many	Sm	+	-	+++
		Some	Sm	+	-	+++

ANIMAL MODELS: WEAKNESSES

- Limits in extrapolating animal to human responses.
- Costs and ease of using a particular species (handling, husbandry & size) exist.
- Availability of reagents can be limited.
- Oral tolerance may alter responsiveness.
- Immune responses may require adjuvants which can limit experimental design to approximate food exposure in humans.

Animal Models: Strengths

- Unlike in man, sensitization (exposure route, dose, timing, and elicitation) can be precisely controlled and standardized.
- Response elicitation, unlike man, can be performed when reactions are most acute and precisely measured.
- Strain selection can help minimize variability in the allergic model.

Rodents: Most likely candidate for assessing novel proteins

- Can easily be tested & skilled personnel readily available in most laboratories.
- An adequate supply of species available.
- Numerous reagents are developed.
- Size provides easier manipulation and reduced cost of housing and care.
- Large database on major histocompatibility complex & the genetics of the immune response exists.

RODENTS

- Brown Norway rats
- Sprague-Dawley rats
- Balb/c Mice
- C3H/HeJ Mice

CHALLENGES BEFORE US

- Rodent responses differ from humans
 - oral tolerance
 - clinical symptoms.
- Can the rodent model predict allergenicity if it does not replicate every aspect of human allergy?
- How to develop a standard animal model of allergenicity from the different ones that are already established?

ESTABLISHING AN ANIMAL MODEL

- **SENSITIZATION CRITERIA**
- IMMUNIZATION PROTOCOL
- ALLERGENIC PARAMETERS TO BE MEASURED
- REQUIREMENTS - STANDARDIZED & VALIDATED ANIMAL MODEL
- THE PRECISION OF THE MODEL TO PREDICT ALLERGENICITY

SENSITIZATION CRITERIA

- Demonstration that animals can be sensitized to known allergens & generate a broad dose-dependent response.
- Conversely it is essential that non-allergens trigger a minimal or negative response.
- The relative potency of an allergen as it is known in humans should be reflected by its order of potency in the animal.

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IMMUNIZATION PROTOCOLS

- Route, number of times, time interval, & dose of sensitizing material.
- Immunizing Allergens - Purified proteins vs protein within the food matrix.
- Test procedures & materials must be well characterized and standardized.

IMMUNIZATION PROTOCOL

- Use of adjuvants to enhance the IgE response is acceptable as long as recognition of allergens vs non-allergens remains different.
- There is the risk of enhancement of responses to non-allergens to a degree that produces false positive results.
- Thus a balance is required - the model must recognize allergens and not recognize non-allergens in a pattern similar to humans.

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Allergenic Parameters to be measured

- IgE or IgG Antibody production, mediator release, or T cell responses
- All must be considered in the context of characterizing allergenicity of a broad range of allergenic or non-allergenic proteins.
- For IgE Antibody production, should recognize known allergenic proteins/major allergens in foods.

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Requirements for a Standardized and Validated Animal Model

- Accuracy & reproducibility – identify an allergen from a non-allergen, produce the same response reliably over the course of time & among different laboratories.
- For well characterized allergens, the allergic response should be similar relative to that observed for humans.
- Should patterns of reactivity to epitopes be similar to those seen by man, although such reactivity does vary in different subjects?

Mouse IgG and IgE Antibody Responses to Shrimp

- Different adjuvants, immunizations protocols, were tested for antibody production in C3H/HeJ, CBA/J, Balb/c, and C57Bl/6J female mice to peanut and shrimp.
- Gavage of allergen plus Cholera Toxin yielded maximal IgE antibody responses in C3H/HeJ and CBA/J mice

Murine IgE Antibody Responses to Allergenic Non- Allergenic Foods

- Further studies demonstrated significant IgE antibody response to allergenic foods (Shrimp, Peanut, Cashew, and Walnut).
- Minimal or no IgE antibody responses were demonstrated to non-allergenic foods (Rice, Beef, Chicken).
- Western Blot analysis of reactivity to Peanut or Shrimp allergens of murine sera from immunized animals demonstrated IgE reactivity similar if not identical to that of peanut or shrimp allergic subjects.

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The precision to predict allergenicity

- Ideally, the animal should respond in a quantitative fashion with less potent allergens proportionally less reactive than more potent allergens.
- However, it may only allow a separation of allergenic & non-allergenic foods such as peanuts and shrimp from peas and beef.
- The dose eliciting a significant response may be a means of sorting out allergenic activity.

Standards and Reproducibility

- Any Model will be only as robust as the standards used.
- Positive and Negative Controls.
- Appropriate levels of accuracy - positive is always positive, negative is negative.
- Reproducibility – acceptable levels of variation for inter and intra-assay performance.

Conclusions – Closing the Gap between Expectations and Reality

- It is highly unlikely if not impossible that any one animal model can mimic the entire human allergic process.
- A model providing a basis for measuring IgE stimulation should be acceptable as a relative measure of potential allergenicity.

Conclusions – Closing the Gap between Expectations and Reality

- The Model must be validated, standardized, and accepted by the general allergy community.
- Thus the model should be regarded as a test system rather than a human surrogate, as a tool to place a protein in relative proportion to its potential allergenicity.

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