EPA Intramural and Extramural Research to Improve Capability to Assess the Risks of Allergenicity from Genetically Altered Food

HESI New Methods Workshop
October 24, 2007, Nice, France

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EPA involved because of GMOs, specifically PIPs

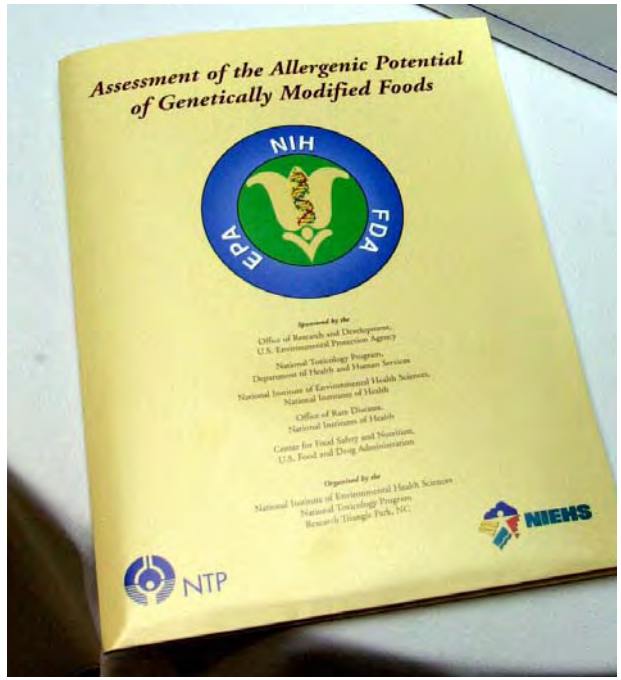
- **Star Link corn episode created the impetus**
- **Current approach to hazard ID**
  - **acid resistant**
  - **homology with known allergens**

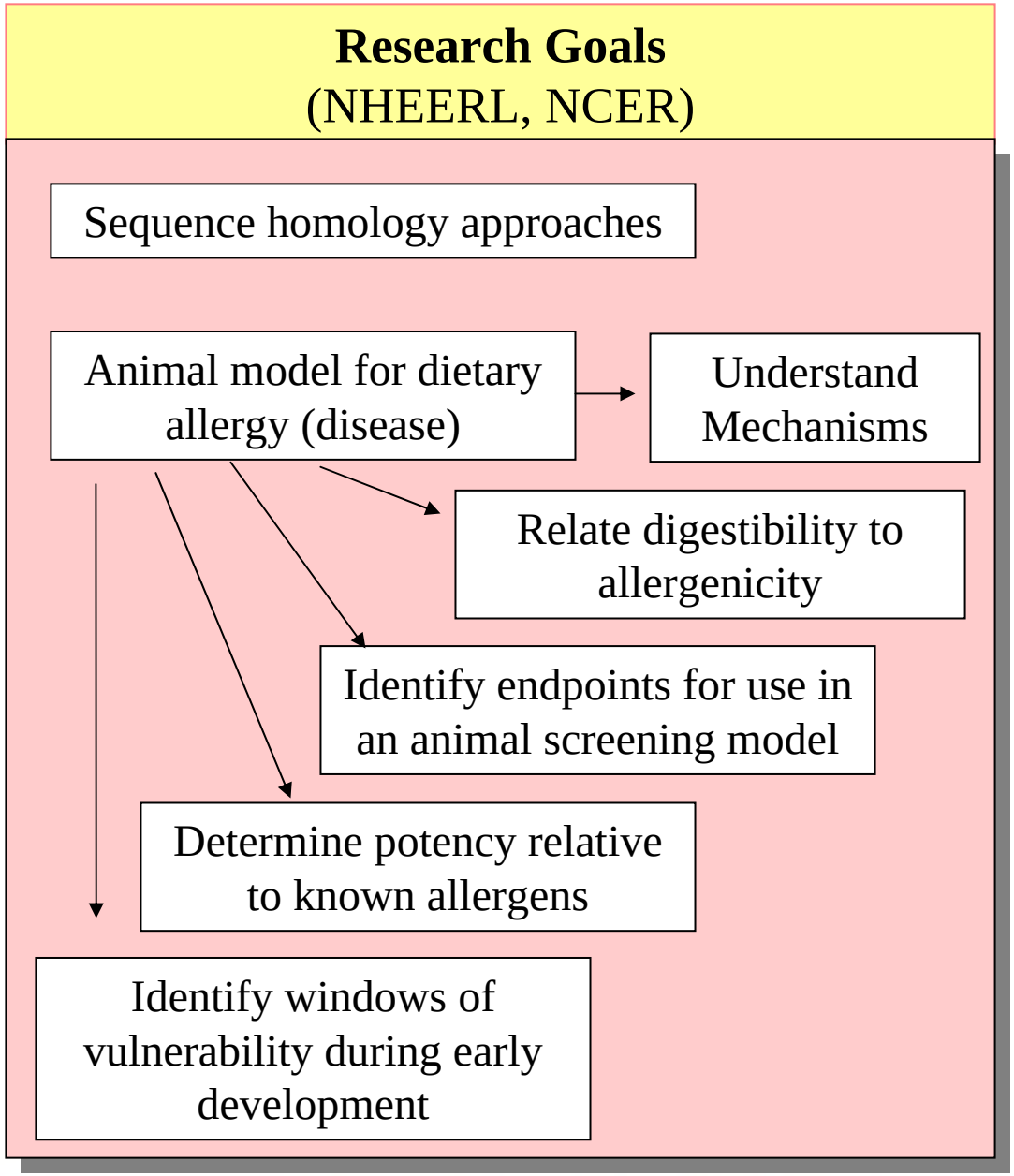
Time Line of Federal Research Events

- **2001 - NIEHS/EPA/FDA workshop –Assessment of Allergenic Potential of Genetically Modified Foods**
- **2002 - EPA Biotechnology Initiative – Including goal to improved capability to assess the risks of allergenicity from genetically altered food**
- **2003 - 1st NIAID Expert Panel on Food Allergy Research**
- **2004 - EPA initiates intramural program**
- **2006 - First EPA RFA and awards**
- **2006 - 2nd NIAID Expert Panel on Food Allergy Research**
- **2006 - USDA hosted meeting to identify research gaps and exploration of opportunities for cross-agency collaborations**
- **2007 - Joint NIAID/EPA RFA**

Workshop Identified Research Needs

- **Develop, evaluate, & validate animal models**
- **Human studies**
  - **Relationships between antigen specific IgE & overt disease**
  - **Susceptibility**
  - **Incidence**
  - **Non IgE allergy**
  - **Systematic recording of adverse events**
- **Better tools**
  - **Human serum banks**
  - **Better skin tests**
  - **Identify, purify, & bank proteins, positive and negative for allergy**




Research Goals
(NHEERL, NCER)
Sequence homology approaches
Animal model for dietary allergy (disease)
Understand Mechanisms
Relate digestibility to allergenicity
Identify endpoints for use in an animal screening model
Determine potency relative to known allergens
Identify windows of vulnerability during early development

Issues Associated with Identifying Allergenic Food proteins

- **Assume something about the protein makes it an allergen**
  - **Is it the protein structure?**
  - **Is it the matrix in which it is presented?**
  - **A little of both**
  - **Digestibility plays a role**
- **Assume potential allergenicity has to do with how the protein interacts with the immune system**
  - **Induction of IgE**
  - **Overcoming oral tolerance**
  - **Must have something to do with dendritic cells and balance between Th1, Th2, Treg**
- **Genetic predisposition**
  - **Unclear what genes are involved, but like asthma probably involves more than one gene, pleiotropic**

Issues Associated with Development of an Appropriate Animal Model

- **Route of exposure**
  - What's most appropriate
  - What's feasible
- **Sensitization regimen**
- **Species and Strain**
  - Mouse, rat, genetically predisposed, knockouts

- **Endpoints and biomarkers of effect**
  - IgE has its limitations
  - Endpoints for tolerance
  - Disease endpoints (anaphylaxis, hives, respiratory)

EPA In-house Research

- **Initiated 2004**
- **Goal to develop one or more animal models**
- **Christal Bowman primarily responsible**
- **Research will be presented tomorrow morning**

First EPA RFA 2006

- **Hazard assessment is of primary interest**
  - **Development and evaluation of animal models for hazard assessment**
  - **Development of targeted or specific serological assays**
  - **Determination of structure-activity relationships of allergenic proteins**
- **Secondary interest - basic issues underlying sensitization to food allergens**
  - **Genetic, developmental and other determinants of susceptibility to food allergy**
  - **Mechanisms underlying food allergy including the development of oral tolerance**
  - **Influence of route, duration and timing of dietary exposure on development of allergenic sensitization**

Four Grants Funded

Risk Assessment of Food Allergenicity by a Data Base Approach

- **Werner Braun, Randall Goldblum, and Catherine Schein, University of Texas Medical Branch at Galveston**
- **Overall goal – further development of structural database of allergenic proteins**
  - **Develop methods for rapidly identifying the locations and structural characteristics of conformational IgE epitopes**
- **Dr. Braun will be speaking later this morning**

Delineation of Appropriate Specific and Targeted IgE Serum Testing to Assess the Potential Allergenicity

- Richard Goodman, Chen Lingyun, Vicki Schlegel, and Steve Taylor, University of Nebraska, Lincoln
- Objectives 1: Devise comprehensive strategy and model protocols to identify specific IgE binding to proteins with various sequential, conformational or glycan epitopes
- Objective 2: Demonstrate the range of IgE binding/cross-reactivity results that can be expected from specific and narrowly defined targeted serum screening.
- Dr. Goodman will be presenting tomorrow afternoon

Safety Assessment of Dietary Proteins for Allergenicity Using an Adjuvant-Free Mouse Model

- **Venu Gangur and Robert Tempelman, Michigan State University**
- **Objective 1: Determine the positive predictive value of mouse based, adjuvant free, transdermal food protein sensitization method**
- **Objective 2: Determine negative predictive value of this model**

Gangur Food Extracts

- **Allergens**
  - Peanut
  - Tree nut
  - Egg
  - Fish
  - Wheat
  - Soybean
  - Shellfish
  - Celery root
  - Mustard seed
  - Sesame seed
  - Milk
- **Non-allergens**
  - Spinach
  - Pinto bean
  - Vanilla
  - Blueberry
  - Kidney bean
  - Mung bean
  - Pigeon pea
  - Amaranth
  - Quinoa
  - Rape seed
  - Sorghum

Gangur Model

- **Sensitization**
  - 5, 50, or 500 ug/100 ul saline applied to back (hair clipped) with non-latex bandage for 3 days
  - Rest 4 days; repeat cycle for 6 weeks
  - Oral Challenge
- **Endpoints**
  - Specific IgG1, IgG2a, IgE and Total IgE
  - Clinical scoring for anaphylaxis
  - Histopathology gastrointestinal tract
- **Reference**
  - Birmingham NP, Parvataneni S, Hassan HM, Harkema J, Samineni S, Navuluri L, Kelly CJ, Gangur V. An adjuvant-free mouse model of tree nut allergy using hazelnut as a model tree nut. Int Arch Allergy Immunol. 2007;144:203-10.

Improved Animal Model for Assessment of Allergenic Potential Foods Through Selective Deletion of T cells and Global Gene Expression Analysis

- **Harm HogenEsch and William Muir, Purdue Univ.**
- **Objectives**
  - **Determine if mice that lack TCRγδ+ T cells (important in oral tolerance) are more sensitive to food allergy**
  - **Determine if oral administration of food proteins to TCR γδ + deficient mice can discriminate between allergenic and non-allergenic proteins**
  - **Identify early biomarkers of food allergy in mice that can shorten exposure protocol and increase sensitivity of our model**

HogenEsch Preliminary Findings

- **Sensitization regimen : via oral feeding tube daily for 5 days or 5 + 5 days (weekend break)**
- **The TCR γδ -deficient mice on a C57BL/6 background;don't produce much IgE regardless of the TCRgd-deficiency.**
- **Two studies with BALB/c mice treated with antibodies also don't show an effect of TCR γδ -depletion.**
- **About to start a study in C3H/HeJ mice with monoclonal antibodies since these mice are thought to be more susceptible to food allergy.**

First round of grants were initiated November 2006

2nd RFA –Joined with NIAID

- **Exploratory Investigations in Food Allergy (R21)**
  - Maximum funding period 2 years
- **Key dates**
  - Released August 23, 2007
  - Letters of intent – November 9, 2007
  - Application due date – December 10, 2007
  - Earliest anticipated funding – August 2008
- **Sponsors and support**
  - NIAID - $2.5 million – 8-10 new awards
- **Purpose**

  High impact, innovative, exploratory/developmental investigations to determine mechanisms of and risk factors associated with IgE-mediated food allergy and related co-morbid conditions, focusing on ex vivo studies with human specimens and studies with current or new animal models of food allergy

Research of interest primarily to NIAID (2007 R21)

- Pathogenesis of food allergy, including mechanisms of allergen processing and presentation to specific T cells, T cell activation, and role of the innate immune system
- Pathogenesis of severe food allergy, including role of mediators, and of pathophysiologic responses in airways or other relevant organs
- Relationship between IgE-mediated food allergy and co-morbid conditions, such as atopic dermatitis, asthma and eosinophilic gastroenteritis
- Genetic components of human food allergy and of severe food allergy

Research of interest to both EPA and NIAID (2007 R21)

- Biomarkers of human food allergy and of the severity of food allergic reactions
- New food allergens, including non-protein allergens and post-translationally modified protein allergens
- Immune mechanisms that underlie the loss of oral tolerance and result in the development of food allergy
- Food allergen epitopes and their relationship to food allergy
- Genetic and environmental (e.g., microbial flora) factors, and their interactions, that modulate sensitization versus tolerance to food

Research of interest primarily to EPA (2007 R21)

- Methods for assessing the risks associated with introduction of novel proteins into the food supply (including animal models, *in vitro* assays, and computational methods)
- Biomarkers of exposure or effects of novel proteins in the diet of existing human or animal populations

Joint Review Process

- **Peer review will be handled through NIAID contractor**
- **EPA will have input into peer review panel**
- **After science peer review proposals will be divided between Agencies and EPA will do relevancy review and subsequently fund some proposals**

Ultimate Goal –Relate Potency of GMO Protein to Other Food Proteins