UNC SCHOOL OF MEDICINE

DEPARTMENT OF PEDIATRICS


# Molecular Characterization of Food Allergens

*Wesley Burks, M.D.*
*Curnen Distinguished Professor and Chair*
*Department of Pediatrics*
*University of North Carolina at Chapel Hill*

UNC North Carolina CHILDREN'S HOSPITAL

# Objectives

- (1) Discuss approaches and techniques used to characterize food allergens
- (2) Discuss cutting-edge applications under development for improving diagnostic and therapeutics based on allergen characterization

# Background: Food Allergy

- Ingestion of foods in an allergic patient can cause range of symptoms mediated by allergen-specific IgE
  - Oral itching, hives, GI discomfort, fatal anaphylaxis
- 4-6% of U.S. children have food allergies
  - Similar prevalence in Europe, Canada, Australia
  - Increase in prevalence of 18% from 1997-2007 in U.S.
  - May be outgrown or persist into adulthood
- Current standard of care is limited to avoidance of the food
- Identification and characterization of food allergens may improve diagnostic and therapeutic approaches

# Food Allergen Classes

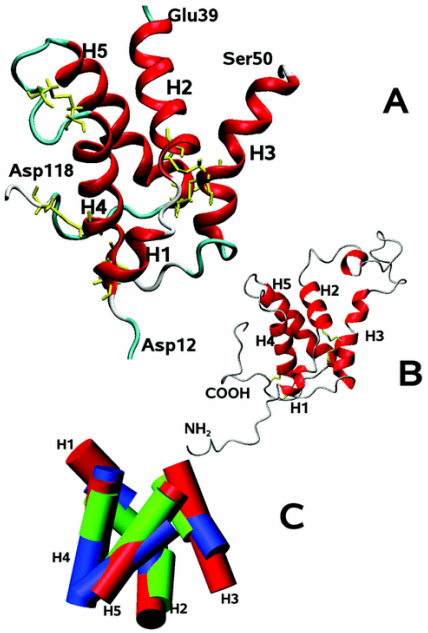
- Classification based on allergen characteristics, with differences in severity of allergic reactions upon ingestion
- Class 1 food allergens
  - 10-70 kD proteins
  - Sensitize and can cause severe, systemic reactions through the GI tract
  - Often resistant to protease digestion, heat, and acidic environments
  - Some form multimeric complexes, such as dimers and trimers
- Class 2 food allergens
  - Occur following sensitization to inhalant allergens (e.g. Bet v 1 from birch pollen)
  - Homologous proteins in fruits and vegetables cause local, oral symptoms
  - Susceptible to protease digestion
  - “Oral Allergy Syndrome”

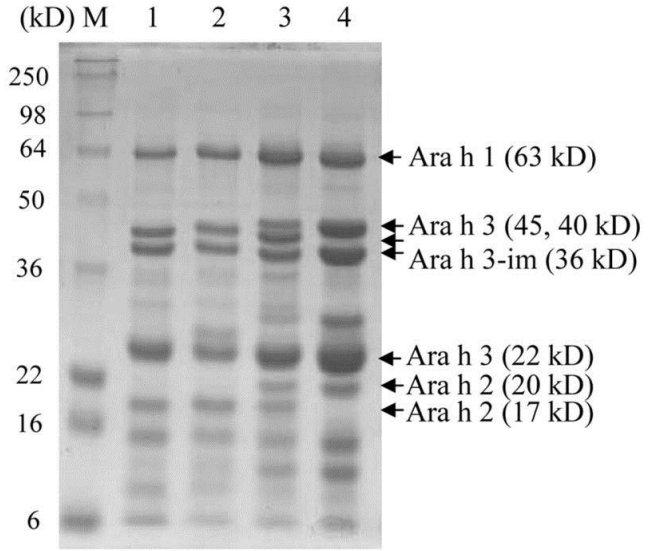
# Overview: Food Allergens

- Allergens from Plant-derived foods
  - Major Sources: Peanuts, Tree Nuts (e.g. Walnuts), Soybean, Wheat
  - Examples of Class 1 allergens:
    - 2S albumins, non-specific lipid transfer proteins, vicilins, legumins
  - Examples of Class 2 allergens:
    - Profilins, Bet v 1 homologs found in fruits, vegetables, and seeds
- Allergens from Animal-derived foods
  - Major Sources: Eggs, Cow's Milk, Shellfish (e.g. Shrimp), Fish
  - Examples:
    - Beta-lactoglobulin, caseins from milk
    - Ovomucoid, ovalbumin from eggs
    - Parvalbumins from fish
    - Tropomyosins from shrimp

# Molecular Characterization

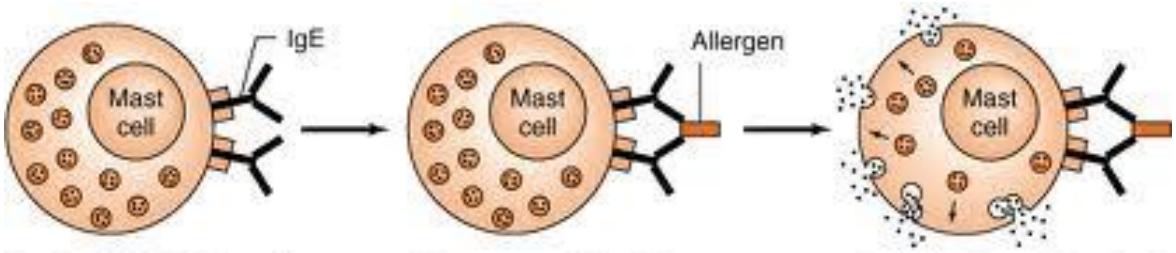
- Allergen Source
  - Taxonomic Order, Family, Genus, and Species
  - Allergen name is derived from genus and species
- Biochemistry
  - Biochemical name of the protein (e.g. tropomyosin)
  - Molecular weight of mature protein
  - Post-translational modifications (e.g. glycosylation)
- Molecular Biology
  - Nucleotide sequence
  - Protein sequence
  - Structure of the protein



# Molecular Characterization

- Allergenicity
  - Define the study population (age, geographical location, etc.)
  - IgE binding assays – Western blot, ELISA
  - Functional assays to demonstrate IgE cross-linking
    - Basophil activation – *ex vivo* from human cells, or rodent cell lines primed with human IgE
    - Skin prick testing
  - Cross-reactivity with homologous protein allergens



# Natural vs. Recombinant Allergens

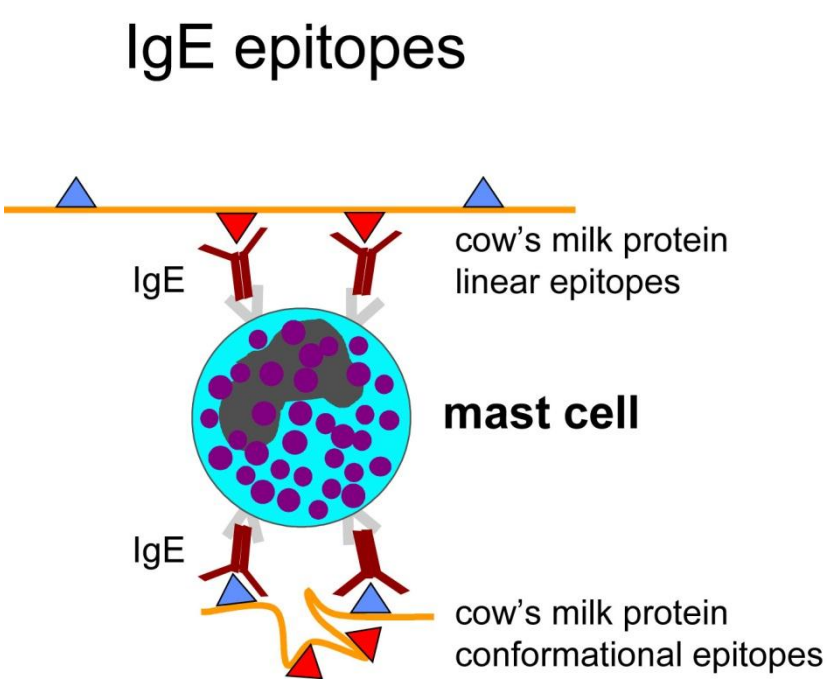
## Natural

- Purified directly from the allergen source (e.g. Peanut)
- Post-translational modifications preserved
- Structure should remain intact

## Recombinant

- Expressed in E. coli, yeast, insect cells, etc.
- Post-translational modifications lost in some systems
- Structure may be altered depending on refolding process
- **Can manipulate protein sequence through site-directed mutagenesis**

# Structural vs. Linear Epitopes




- Class 1 food allergens often have linear epitopes that persist after digestion
- Recognition of conformational epitopes may indicate transient allergy as demonstrated for outgrowing egg allergy[1]
- Class 2 food allergens have conformational epitopes that are destroyed on digestion leading to only local, oral symptoms

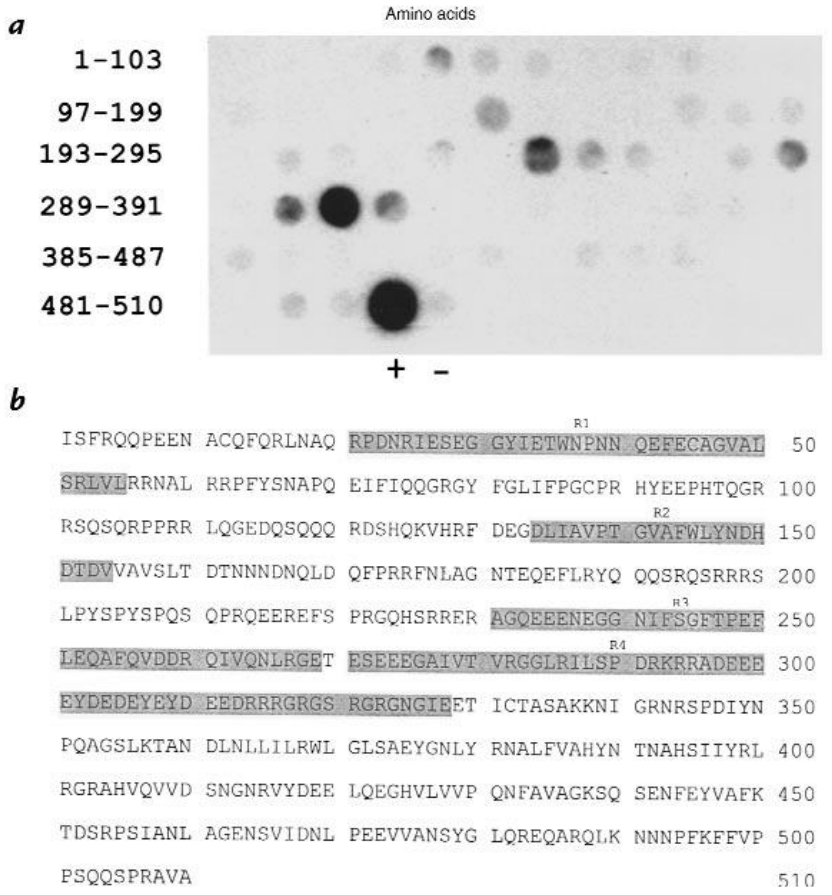


1. Jarvinen et al. Allergy 2007

# IgE Epitope Identification

- Synthetic peptides are coupled to a membrane (SPOTs)
- 10-15 amino acid peptides
- Overlap of 3-5 amino acids, spanning the entire protein sequence
- Probed with human IgE from allergic patients
- Immunodominant epitopes in a population can be identified

Peanut Legumin Allergen, Ara h 3

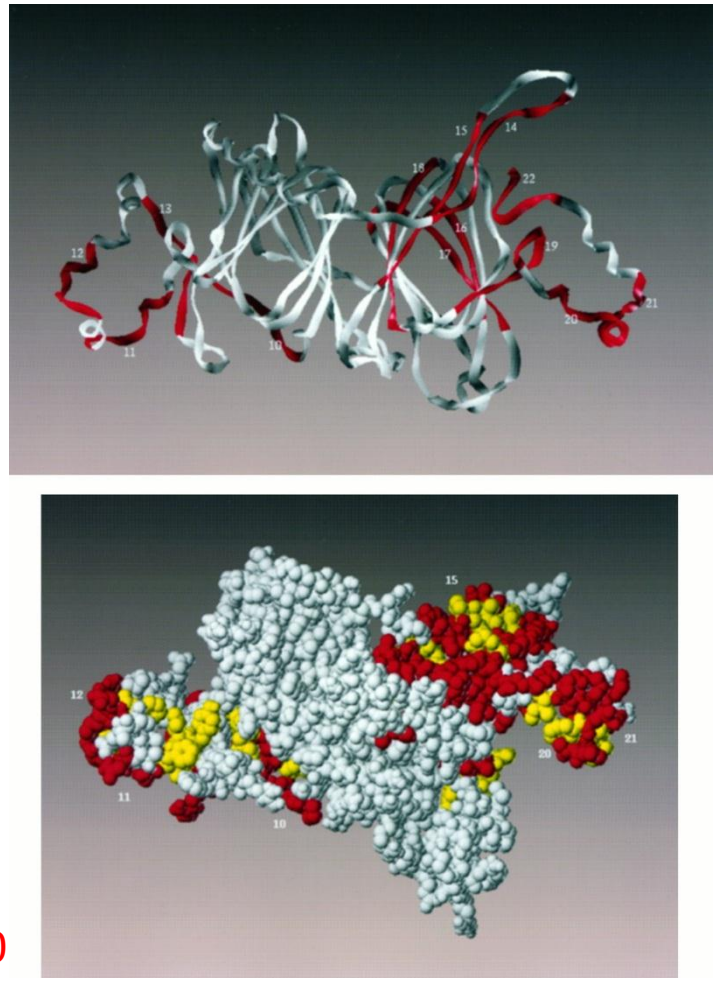



Rabjohn et al. J Clin Invest 1999

# IgE Epitopes in 3D Space

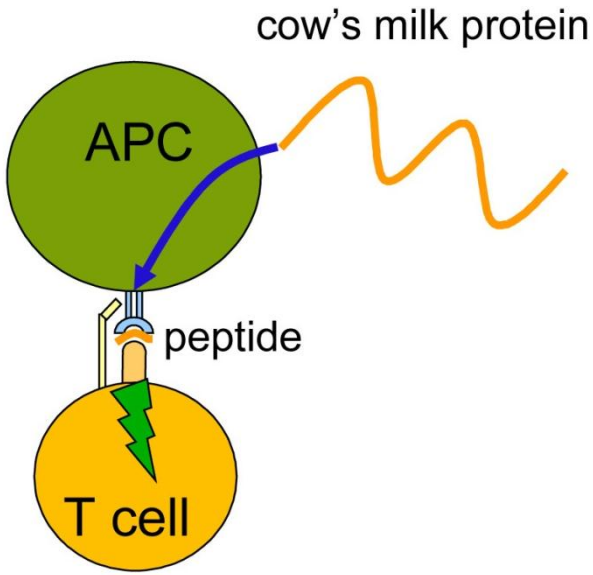
- IgE epitopes discovered through synthetic peptides can be mapped on 3D structures of allergens
- Gives insight into “clustering” of epitopes and how these may interact with mast cells or be protected during digestion

Peanut Vicilin Allergen, Ara h 1



Maleki et al. J Immunol 2000

# T Cell Epitope Identification




- More difficult than IgE epitope analysis
- T cell epitopes are dependent on HLA-type
- Requires live cells and readouts such as proliferation and cytokine production
- Substantially less information available regarding T cell vs. IgE epitopes
- Recent progress on this front has led to the development of Ara h 1 **Tetramers**[1]
- Tetramers can be used to phenotype and isolate food allergen-specific T cells



1. DeLong et al. J Allergy Clin Immunol 2011

# Novel Diagnostic Approaches

- Component-resolved analyses
  - Following isolation and characterization of food allergens, we can determine which proteins bind IgE in specific patients
  - IgE against particular allergens may lead to distinct phenotypes

- Peanut allergy
  - Typically diagnosed with peanut extract (e.g. Phadia ImmunoCAP)
  - Peanut-specific IgE > 15 kU/L indicates 95% certainty in predicting clinical reactivity
  - However, many patients are sensitized but will not react
  - Components now available: Ara h 1, 2, 3, 8, 9

# Component Data

- Peanut allergy
  - Ara h 2 appears to be the most informative for clinical reactivity[1]
    - 81 children in the U.K. with detectable peanut-IgE underwent DBPCFC (29 reacted, 52 tolerant)
    - Ara h 2 > 0.35 kU/L correctly classified 97% of subjects
    - Peanut > 0.35 kU/L correctly classified 51% of subjects
  - Ara h 8, the Bet v 1 homolog in peanut, indicates sensitization to peanut, but with mild oral-allergy syndrome[2]
  - Ara h 9, the peanut lipid transfer protein, is relevant in certain geographical locations (i.e. Mediterranean)[3]



1. Nicolaou et al. JACI 2011
2. Asarnoj et al. JACI 2012
3. Vereda et al. JACI 2011

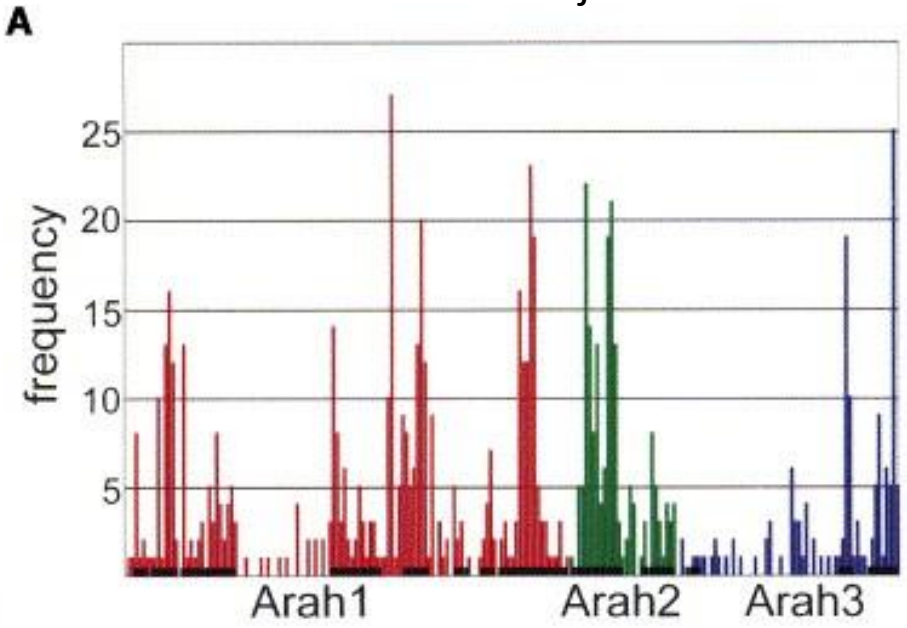
UNC SCHOOL OF MEDICINE

DEPARTMENT OF PEDIATRICS

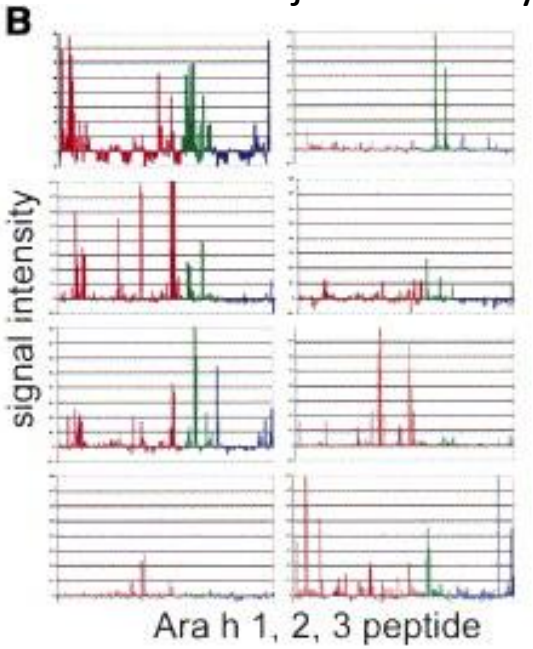
# Epitope Arrays

- Synthetic peptides are coupled to a glass slide
- Overlapping peptides, span the entire protein sequence
- Binding of IgE and IgG4 to peptides is determined with < 100 uL serum

Results from n=77 subjects




Individual subject diversity




Shreffler et al. JACI 2004

North Carolina CHILDREN'S HOSPITAL

# Epitope Arrays

- Utility of IgE and IgG4 epitope binding patterns
  - Milk allergy phenotypes[1]
    - IgE recognition to broad range of epitopes is associated with milk allergy, whereas smaller repertoire is associated with heated milk-tolerant subjects, and those that have outgrown milk allergy but remain sensitized
  - Peanut allergy clinical reactivity[2]
    - Bioinformatic approach identified 4 peptides able to predict DBPCFC outcomes in allergic and sensitized-but-tolerant subjects
  - Changes induced by peanut oral immunotherapy[3]
    - Peptide-specific responses of IgE and IgG4 were studied indicating isotype switching from IgE to IgG4 at some peptides, while IgG4 developed *de novo* to other peptides

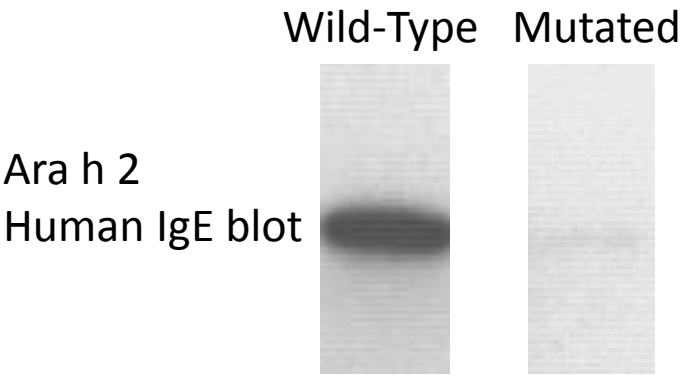


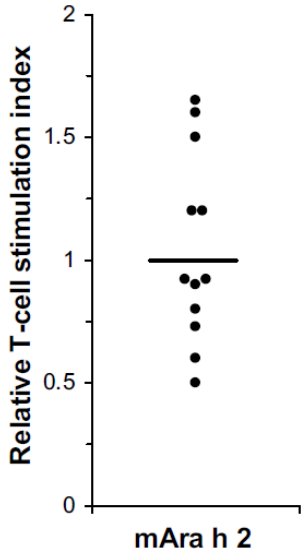
1. Wang et al. JACI 2010
2. Lin et al. JACI 2012
3. Vickery et al. JACI 2012

# Therapeutic Approaches

- Currently, clinical trials for food allergy involve crude antigen preparations, such as in OIT and SLIT
- Manipulation of individual allergens may improve clinical applications

- Preclinical assessments using *mutated allergens* show promise
  - Mutate IgE binding epitopes while preserving T cell epitopes
  - Mutated Ara h 1-3 can effectively treat peanut allergy in a mouse model




King et al. Mol Nutr Food Res 2005

# Therapeutic Approaches

- Preclinical assessments using *allergen peptides* as immunotherapy
  - Determination of T cell epitopes for various HLA-types
  - Small peptides that will not cross-link IgE (e.g. 15-mer)
  - Can drive T cell responses without causing allergic side effects

Healthy controls

Peanut allergic

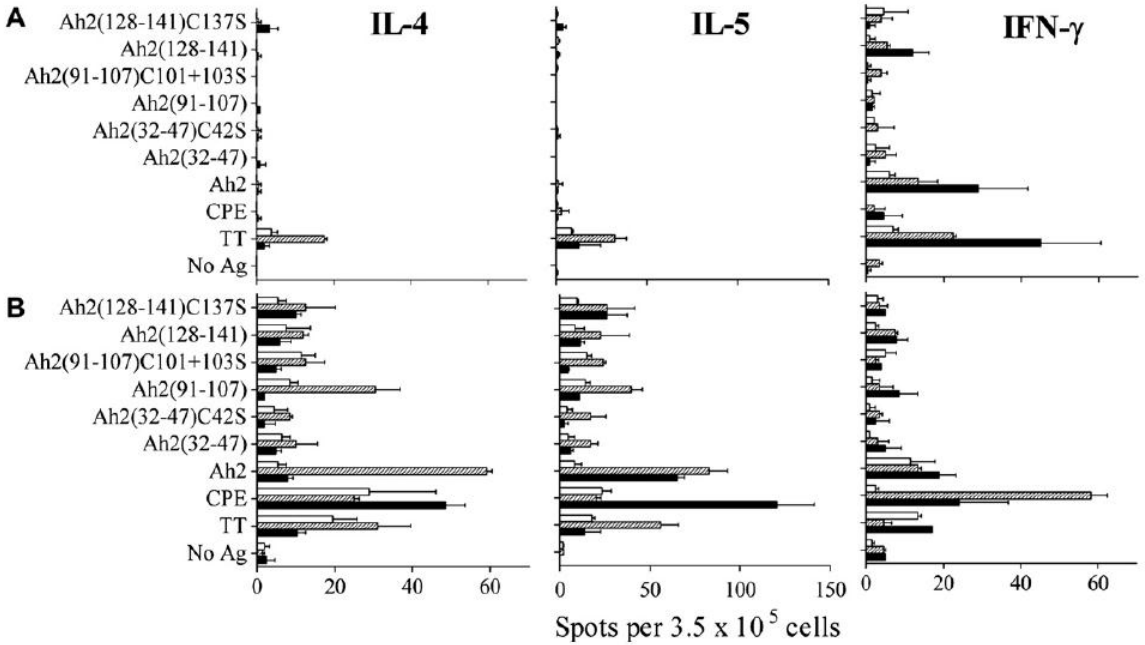



FIG 6. PBMC cytokine secretion in response to T-cell epitope-derived peptides. PBMCs from 3 nonatopic control subjects (A) and 3 subjects with peanut allergy (B) stimulated with CPE, nAra h 2, candidate peptides, or TT (control). IL-4, IL-5, and IFN-γ secretion determined by ELISPOT. Mean spots of replicate wells (+SD) shown for each subject. *Ag*, Antigen.

Prickett et al. JACI 2011

# Conclusions

- Many food allergens have been identified and characterized at the molecular level
- While some common features exist, it is not clear why some food proteins are allergens and others are not
- Exploiting our current understanding of these proteins may lead to better diagnostic and/or therapeutic approaches in the future

UNC SCHOOL OF MEDICINE

DEPARTMENT OF PEDIATRICS

# Thanks

## Grant support

*Food Allergy and Anaphylaxis Network, Food Allergy Project, Gerber Foundation, NIHR01 – AI, NIHR01-NCCAM, NIH 1 UL1 RR024128-01 (DCRU), Robbins Foundation*

## Team

- Physicians - Joe Roberts, Brian Vickery, Stacie Jones (AR), Hugh Sampson (Mt. Sinai), Wayne Shreffler (Harvard), Edwin Kim (UNC)
- Study coordinators - Pam Steele, Jan Kamilaris, Michele Cox
- Fellows - Amy Scurlock, Arianna Buchanan, Todd Green, Scott Nash, Pooja Varshney, Ananth Thyagarajan, and Drew Bird
- Laboratory - Xiaoping Zhong, MD/PhD; Laurent Pons, PhD; Mike Kulis, PhD; and Herman Staats, PhD

UNC North Carolina CHILDREN'S HOSPITAL