Molecular Characterization of Food Allergens

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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
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.

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

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.

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

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
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
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 \textit{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

[Image: Wild-Type Mutated Ara h 2 Human IgE blot]

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

![Graph showing 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.](image)
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
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Team

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