Allergy Diagnostics and Immunotherapy

Molecular Allergy Diagnostics
*What, Why, Who, When, How*

Robert G. Hamilton, Ph.D., F.AAAAAI
Course 1211, February 22, 2013

Disclosures:

- Research Projects: Thermofisher Scientific, Siemens Healthcare
- Scientific Advisory Board: Hycor Biomedical
Objectives

- Discuss the “4 Ws and H” of molecular allergy diagnostics (What, Why, Who, When and How)

- Update on developments in serological methods used in the diagnosis and management of allergic disease

Principal Analytes Measured in Diagnostic Allergy Laboratories

- Allergen-specific IgE (over 200 allergen specificities)
  - Pollen (weeds, grasses, trees), Epidermals, Dust Mites, Molds, Foods, Venoms, Occupational allergens, Drugs

- Total Serum IgE (Xolair: anti-IgE; ABPA)

- Mast Cell Tryptase (indicator of anaphylaxis)

- Precipitating IgG Antibody (Hypersensitivity Pneumonitis)

- Eosinophil Cationic Protein (eosinophil activation marker)
What do we mean by molecular diagnostics?

Component Resolved Diagnosis

Diagnosis of Human Allergic Disease
Use of Allergen Extracts

19th century: Blackley: pollen grains were the cause of hay fever by rubbing pollen grains into a "break in skin" on his arm, producing the "same reaction" as hay fever

1911: Noon and Freedman credited with introducing skin test into Britain
Schloss and Walker introduced the scratch test modification into the USA
British and Europeans developed the prick test variant which Pepys perfected (1950s)
Allergen Extracts Used For Diagnosis and Immunotherapy in USA

19 standardized allergen extracts controlled for potency and stability
  D. farinae, D. pteronyssinus, cat hair, cat pelt, short ragweed,
  Hymenoptera (HBV, PWV, YJIV, YHV, WPHV, mixed vespid venom),
  Grass pollens (Bermuda, red top, June, perennial rye, orchard timothy,
  meadow fescue, sweet vernal)

1269 non-standardized allergen extracts: no defined potency, composition, stability (expiration date?)

FDA Allergen Efficacy Review

Internal FDA Review 2004-2006: >1500 allergen extracts
1269 non-standardized extracts: Animal: 28, Molds 180, Dusts: 6,
  Plants 16, Foods 277, Pollens: 708, Insects: 34

Table 1: Use in Diagnosis and Treatment Addressed in Literature (480)
Table 2: Food: Use in Diagnosis Addressed in Literature (134)
Table 3: Non-food: Use in Diagnosis Addressed in Literature (73)
Table 4: Minimal/no literature related to Diagnosis or Treatment (566)
Table 5: Potential Safety Issues (16): e.g. house dust, monkey pelt

Diagnosis of Human Allergic Disease
Allergen Extract Based Serological Assays


Allergen Extract Based Diagnostic IgE Antibody Tests

Inhalants
- Tree Pollen
- Weed Pollen
- Grass Pollen
- Epidermals
- Insects
- Mites
- Molds

Injected Venoms-Drugs

Ingestants

Foods
Diagnosis of Human Allergic Disease
Evolution from Allergen Extracts to Components

Application of purified component allergens into IgE antibody assays

Purified molecular allergens (recombinant [r] and native [n])

Peanut (Arachis hypogaea) Ara h 2
Genus Species #
Early (FDA cleared) Components available on Singleplex Autoanalyzers

* Food: (cow’s milk) nBos d 4,5,6
  - (α-lactoalbumin; β-lactoglobulin; casein)
* Food: (chicken egg) n Gal d 1,2
  - (ovomucoid, ovalbumin)
* Occupational (α-amylase) n Asp o 21 (Aspergillus oryzae)


Cross-reactive Allergen Families of Clinical Significance

- Tropomyosin
- Serum Albumin
- Non-specific Lipid Transfer Proteins
- Pathogenesis Related Proteins: PR10 Family (Bet v 1 homologues)
- Profilin
- Thaumatin-Like Protein
- Carbohydrate Cross-reactive Determinants
Cross-reactive Allergen Families

- **Tropomyosin**: Actin-binding muscle protein that regulates actin mechanics in muscle contraction: Anisakis-Ani s 3; Cockroach-Bla g 7; Dust mite-Der p 10; Shrimp Pen m 1

- **Serum Albumin**: Protein that functions to transport fats and fatty acids to muscle tissue: Cow-Bos d 6; Dog-Can f 3; Horse-Equi c 3 Cat -Fel d 2

- **Non-specific Lipid Transfer Proteins**: plant proteins that shuttle phospholipids/fatty acids between cell membranes
  - Peanut-Ara h 9; Hazelnut-Cor a 8; Walnut-Jug r 3; Peach-Pru p 3; Mugwort -Art v 3; Olive Pollen-Ole e 7; Plane tree -Pla a 3

Cross-reactive Allergen Families

- **Profilin**: An actin-binding protein involved in the dynamic turnover and restructuring of the actin cytoskeleton
  - Birch -Bet v 2; Natural Rubber Latex -Hev b 8; Mercury-Mer a 1; Timothy grass -Phl p 12
  - **Thaumatin-Like Protein**
  - Green Kiwi - Act d 2

- **Carbohydrate Cross-reactive Determinants**:
  - Bromelain (pineapple)-MUXF3: sensitization via pollen, insect venom
  - Galactose-α-1,3 galactose-sensitization via tick bites, parasites?
Pathogenesis Related Proteins: PR10 Family (Bet v 1 homologues)-Ribonuclease

<table>
<thead>
<tr>
<th>Pollen</th>
<th>Pomaceous and stone fruit, nuts</th>
<th>Vegetables/ legumes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aln g 1 (alder)</td>
<td>Act c 8 (gold kiwi)</td>
<td>rApi g 1 (celery)</td>
</tr>
<tr>
<td>rBet v 1 (birch)</td>
<td>Act d 8 (kiwi)</td>
<td>rAr a 8 (peanut)</td>
</tr>
<tr>
<td>Car b 1 (hornbeam)</td>
<td>Cas s 1 (sweet chestnut)</td>
<td>Arpa o 17KD (asparagus)</td>
</tr>
<tr>
<td>Cas s 1 (sweet chestnut)</td>
<td>rCor a 1 (hazelnut)</td>
<td>Dau c 1 (carrot)</td>
</tr>
<tr>
<td>rCor a 1 (hazel)</td>
<td>Fra a 1 (strawberry)</td>
<td>rGly m 4 (soy)</td>
</tr>
<tr>
<td>Fag s 1 (beech)</td>
<td>Mal d 1 (apple)</td>
<td>Pet c 1 (parsley)</td>
</tr>
<tr>
<td>Que a 1 (oak)</td>
<td>Pru ar 1 (apricot)</td>
<td>Vig r 1 (mungbean)</td>
</tr>
<tr>
<td>rPru p 1 (peach)</td>
<td>Pru av 1 (cherry)</td>
<td></td>
</tr>
<tr>
<td>Pyr c 1 (pear)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rub i 1 (raspberry)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Treurder R., Update on in vitro allergy diagnostics. JDDG, 10:89-99, 2012

FDA Cleared Food Allergen Components (as of 2013)  
Available on Singleplex Autoanalyzers

- Peanut:  rAra h 1, 2, 3, 8-PR-10, 9-LTP
- Hazel nut:  rCor a 1-PR-10, 8
- Brazil nut:  rBer c 1
- Shrimp:  rPen a 1-tropomyosin - nPen m 1 -tropomyosin
- Peach:  rPru p 1-PR-10, 3-LTP, 4-profilin
- Soy:  rGly m 4-PR-10, n5, n6
- Olive:  rOle e 1, 7 –LTP, 9
FDA Cleared Inhalant Allergen Components (as of 2013)

- Birch: \( r/n \) Bet v 1-PR-10, 2-profilin, 4,6
- Timothy grass \( rPhl \ p 1, 2, 5b, 6, 7, 12 \)
- Bermuda grass \( nCyn \ d 1 \)
- Dust mite \( nDer \ p 1, 2, 10-tropomyosin \ -- nDer f 1, 2 \)
- Alternaria \( rAlt \ a 1 \)
- Cat \( rFel \ d 1, 2, 4 \)
- Dog \( rCan \ f 1, 2, 3, 5 \)
- Horse \( Equ \ c 1 \)
- Ragweed \( nAmb \ a 1 \)

Reviews on Allergen Component based Diagnostics

- Treudler R, Update on in vitro allergy diagnostics. JDDG, 10:89-99, 2012
Why consider molecular (allergen component) over allergen extract based IgE antibody analyses?
Dynamic Factors That Alter the Degree of Sensitization

Patient Factors
- age (child vs adult), gender, race, social economic status, family atopy history-genetic predisposition, immune status

Environmental Factors
- allergen source (complexity-concentration), duration and route of exposure, environment, pet ownership, smoking

IgE Antibody Quantity/Quality Changes
- Concentration (kUa/L)
- Affinity (tightness of binding) Ka/Kd
- Clonality (epitope specificity)

Specific Activity (specific IgE to total IgE ratio)
Christensen et al JACI;122:298-304, 2008

Advantages of Allergen Extract use in IgE Antibody Assays

Practical issue: extracts of biological materials are easier to prepare

Physiological extracts (in theory) contain the most comprehensive profile of allergens of clinical relevance achievable for that specificity
Problems with Allergen Extract Use in IgE Antibody Assays

- Difficult to standardize complex allergen extracts due to natural variability of allergen sources

- Assays using extracts detect different populations of IgE antibody

- Cannot differentiate between primary sensitization and immunological cross-reactivity

- Cannot predict risk or identify prognostically significant sensitizations

Who (in terms of physician group) might best use molecular methods?

Who (in terms of manufacturers) provides molecular based analyses?
Users of Component Resolved Diagnosis

- **Pediatric Allergist** – Yes, only for restricted allergen components (e.g. peanut); Need unique CPT code to allow reimbursement.

- **Adult Allergist** – rarely, at least until cost is addressed or for complex multi-sensitized patients

- Other clinicians (primary care, ENT) – No

---

**When** (what clinical conditions) are molecular analyses particularly useful over extract based analyses?
Strengths of Allergen Component based IgE Measurements

- Optimizing analytical sensitivity by replacement or supplementation of relevant allergens in test extracts (*Hevea brasiliensis*-Hev b 5; hazelnut-Cor a 1)
- Standardization of allergen extracts using individual allergens
- More precisely defining sensitization profiles for complex patients sensitized to many allergen groups
- Detection of cross-reactions and predictive risk factors (e.g., peanut)

Differentiation of Peanut Allergy from Tolerance using Component Resolved Diagnostics

- Children recruited prenatally; evaluated at 1, 3, 5, 8 yrs
- Peanut sensitization identified by PST (>3mm) or ImmunoCAP (>0.2 kUa/L); n=108

1. **Peanut allergic**: Hx of reaction and IgE anti-peanut ≥ 15 kUa/L or PST >8 mm; or failed peanut challenge (n=29)
2. **Peanut tolerant**: sensitized & negative peanut challenge (n=52)

- 10% of 8yr children were sensitized; ~2% peanut allergic
- Microarray component resolved IgE identified Arah2 as the single best discriminator of tolerance vs allergy and the most important predictor of clinical allergy to peanut

Niacolaou N et al. JACI 125:191-197, 2010
Component Resolved Diagnostics Utility
Nicolaou et al. JACI 125:191-7, 2010

- Child, + peanut allergy history, + PST to peanut
- Prognosis (risk for systemic reaction) can be very different if sensitized to
  - Ara h 8 [Bet v 1 (PR10) like protein] {minimal systemic reaction risk {marker for primary sensitization to birch/alder pollen}
  - Ara h 1, 2, 3 [seed storage proteins] high risk {Ara h 2 is considered a risk marker for severe allergic reactions}
  - Ara h 9 [lipid transfer protein] high risk {suggests primary sensitization to peach or other LPT-containing fruits}

How is component specific IgE technically measured?
**FDA-Cleared IgE Antibody Single-plex Autoanalyzers**

Basic reagents and chemistries are similar:
* Allergen on solid phase binds antibody
* Buffer wash to remove unbound protein
* Enzyme anti-IgE detects bound IgE

*All assays report in similar units with comparable analytical sensitivities of 0.1 kUA/L.*
* All assays principally use allergen extracts;*
* Limited use of purified allergens (e.g., insulin)*

---

**Diagnosis of Human Allergic Disease**

**Multiplex Chip Technology Using Allergen Components**

- Characterisation of IgE
- First allergens cloned
- Diagnostic recombinant allergen panels
- ImmunoCAP ISAC 96
- Provocation testing
- RAST testing
- In vivo testing
- In vitro testing
- Computed resolved diagnosis

ThermoFisher Scientific ISAC® Technology

Duration: 3 hours

Future Studies

1. Definitive head to head diagnostic sensitivity/specificity comparison of puncture skin testing with autoanalyzer based IgE antibody tests using provocation testing as the arbiter for allergic disease.

2. Determining the clinical value of IgE antibody levels between 0.1 and 0.35 kUa/L

3. Defining variability and ideal use of predictive kUa/L thresholds for conducting food challenges

4. Harmonizing allergen extracts used across different serological IgE antibody assays.
Trends in IgE Antibody Serology

- Extract Based
  Singleplex Assays with
countification in
kUa/L

IgE Antibody Assays

- Allergenic
  Molecules
  (components)

Multiplex
Chip-Based
ISAC

Diagnostic Algorithm for the Assessment of
Human Allergic Disease

Clinical History & Physical Examination
Symptoms v Exposure
Risk Factors
[Clinical History Drives the Diagnosis]

Diagnostic (Confirmatory Test) for Sensitization (IgE)
Skin Test (Puncture, Intradermal)
Allergen-specific IgE Antibody Serology

Provocation Test
Inhalation, ingestion or injection Challenge
(natural v control exposure)