Allergenic Cross-Reactivity
Concepts, Patterns & Clinical Implications

Overview
- Cross-reactivities: Focus of considerable interest/investigation over last 10-15 yrs
- Coincides w/ significant scientific/clinical advances in allergy and clinical immunology
  - *In vivo* pathways/mechanisms
  - *In vitro* methodologies, Ag-Ab interactions
  - Allergen ID/characterization, Extract properties
  - Genetic/molecular biology developments
  - Clinical measurements of sensitivity and IT efficacy

New developments and insights into the molecular basis of allergen cross-reactions
Diverse/novel approaches, methods and criteria
- Shared vs. distinct Ag/epitope structures
  - Ag repertoires/pt profiles, Characteristic Ag groups
- Relationships, similarities and relevance
- Medline: ~ 500 publications in last 10 years
  - Allergy/immunology + molecular/biochem journals

Challenges
- How to acquire & maintain up-to-date information
- How to understand/interpret data and relationships
- How to establish cross-reactivity patterns pertinent to your patients and practice
- How & when to incorporate cross-reactivities into current testing and treatment regimens

Concepts and Perspectives
- Conservation of protein allergen structures
- Taxonomy genetics vs. biochemistry
- Structure-function-activity relationships
- Clinical vs. immunochemical cross-reactivities
- Allergen-antibody binding requirements

Key Elements
- Patterns vs. # and ID of common components
- Sequence homology vs. structural similarity
- Major allergen definition, ID and importance
- Isoallergens and epitope diversity
- Continuous vs. discontinuous Ag structures
Cross-Reactivity Perspectives
- Clinical
  - IgE specificities in mast cells, basophils and sera
- Taxonomic
  - Conservation and homology of genomes or sequences
- Biochemical
  - Allergen/antigen protein sequences and structures
  - IgE/IgG binding site conformations/topologies

Clinical Cross-Reactivity
- Implied from patient specificity profiles
  - Prick/intradermal skin test relationships
  - Total and allergen-specific IgE levels (CAP/RAST)
  - Exposures, challenges or IT with representative Ags
- Strengths
  - Comprehensive sens/spec patterns for individuals & gps
- Weaknesses
  - Cannot distinguish parallel/independent from shared Ags
  - Implications not always consistent w/ actual pt sens

Clinical Sensitivities
- Single- vs. multiple-allergen sensitivities
  - Not distinguished by history, skin test, sp IgE
  - Often confirmed by cross-wise IgE inhibition IAs
- High CR relevance
  - Pollens, dust mites
- Lower CR relevance
  - Fungi, insects, animals
- Cross-reactive allergens = additive IT doses
  - Diversity/mixes vs. Representation/individual Ags
  - Patient sens/exposures vs. Extract compositions

Taxonomic Cross-Reactivity
- Consistent with phylogenetic relationships
  - Common genus, order, family, subfamily, ...
  - Genetic mapping, Gene/protein sequence homologies
  - Complicated by presence of natural + designed hybrids
- Strengths
  - Patterns consistent w/ pt rxns in many (not all) cases
- Weaknesses
  - Epitopes may include unique, non-homologous regions
  - Sensitive to source material/extract/pt specificity diffs

Biochemical Cross-Reactivity
- Based on structural & immunochemical data
  - Protein sequences/structures, Epitope/domain mapping
  - Compositional fingerprints (IEF, SDS-PAGE, CIE)
  - Immunoassays (ELISA, blot, CRIE) using human/animal Abs
- Strengths
  - Precise Ab specificities, Shared vs. unique 1°, 2°, 3° regions
- Weaknesses
  - IgE specificities & CRs may differ from those found for IgG
  - Complicated by Ag isoforms, multivalent Ags, polyclonal Abs

Conservation of Allergen Structures
- Similar genetic classifications and/or similar protein functions
- Homology
  - Total/local, Exposed/buried, 1°/2°
- Isoforms
  - Amino acids and sugar chains
  - Number + chemical nature of structural differences
  - Conservative vs. non-conservative substitutions
  - Carbohydrate chains (+), sequences and locations
Serine Protease Homology

Molecular Basis of CRs
- Requirements for orientations + interactions of antibodies and specific allergenic proteins
- Complementarity/spatial relationships between 3º (3D) Ag conformations and hypervariable regions of Ab molecules (F_{\text{ab}})
- Number + nature of contact regions essential to high-affinity, multi-point Ag-Ab binding

Allergen-Antibody Interactions
- Antibodies recognize precise arrangements of chemical groups on allergenic proteins
- Secondary/tertiary structures, cofactors, ionic + hydrogen bonds between Ag and Ab
- Includes linear (sequential) and non-linear (remote, conformational) allergen structures

Allergen-Antibody Complexes
- Minimal & optimal combinations w/ binding strengths determined by overall fit/contacts
- Structures and interactions influenced by relatively minor changes in sequence/chem
- Complex formation sensitive to variations in patient specificity, raw material/extract composition, product stability/compatibility

IgG/IgE Structure and F_{\text{ab}} Domain

F_{\text{ab}} \text{ V}_{\text{H}} \text{V}_{\text{L}} \text{ Topography}
MAb-Lysozyme Binding Site

General Patterns of Allergenic Cross-Reactivity

- **Same genus/tribe**: Strong cross-reactions
  - Closely-related compositions and protein structures
- **Same subfamily**: Moderate cross-reactions
  - Similar compositions with different/uniform structures
- **Diff. subfamilies**: Low/no cross-reactions
  - Distinct compositions/structures, minimal similarities

Tree Pollen Cross-Reactivities

- Maple / Box elder (genus Acer)
- Red cedar / Mtn cedar / Juniper (genus Juniperus)
- Cottonwood / Poplar (genus Populus)
- Birch / Alder / Hazelnut (family Betulaceae)
- Oak / Beech / Chestnut (family Fagaceae)
- Non-Juniperus cedars / Cypress (family Cupressaceae)
- Walnut / Hickory / Pecan (family Juglandaceae)
- Olive / Ash / Privet / Lilac (family Oleaceae)

Weed Pollen Cross-Reactivities

- Giant/Short/False/Western ragweed (genus Ambrosia)
- Dock / Sorrel (genus Rumex)
- Sage / Mugwort (genus Artemisia)
- Pigweed / Careless weed (genus Amaranthus)
- Lambs quarter / Mexican tea (genus Chenopodium)
- Marsh elder / Poverty weed (genus Iva)
- Scale / Saltbush (genus Atriplex)

Grass Pollen Cross-Reactivities

- **Strong**
  - Blue/ Fescue/ Rye/ Orchard/ Brome (A)
  - Timothy/ Redtop (B)
  - Sweet vernal/ Canary/ Reed canary (C)
  - Cultivated oat/ Velvet (D)
- **Moderate**
  - Bermuda vs. Salt (E)
  - Bahia vs. Johnson (F)
- **Low**
  - (A/ B/ C/ D) vs. (E/ F)
  - (E) vs. (F)

Prominent Grass Pollen Allergens

<table>
<thead>
<tr>
<th>Group</th>
<th>Activity or function</th>
<th>Mol wt (kda)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Beta-expansin</td>
<td>27-35</td>
</tr>
<tr>
<td>4</td>
<td>High MW alkaline glycoprotein</td>
<td>50-70</td>
</tr>
<tr>
<td>5</td>
<td>Starch granule-assoc. protein</td>
<td>27-35</td>
</tr>
<tr>
<td>7</td>
<td>Calcium-binding protein</td>
<td>8-12</td>
</tr>
<tr>
<td>10</td>
<td>Cytochrome c</td>
<td>11</td>
</tr>
<tr>
<td>11</td>
<td>Trypsin inhibitor (Soybean)</td>
<td>16-18</td>
</tr>
<tr>
<td>12</td>
<td>Profilin</td>
<td>12-14</td>
</tr>
<tr>
<td>13</td>
<td>Polygalacturonase</td>
<td>50-60</td>
</tr>
</tbody>
</table>
**Prevalence of Positive IgE Reactions**

- **Group 1 Grass Allergens**
  - Present in temperate + subtropical species
    - Per. rye Lol p1, Timothy Phl p1, Bermuda Cyn d1
  - Identical runs ≥ 10aa in 250aa Gp1 structures
    - Tim 1 vs. Rye 1: 10 runs 10, 12, 14, 16, 18, 20, 22, 24, 27, 31 aa
      - 24 aa diffs/247 = 9% 91% homology
      - 24 diffs = 11 conserv + 13 non-conserv
    - Ber 1 vs. Rye 1: 1 run 11 aa
      - 76 aa diffs/247 = 31% 69% homology
      - 76 diffs = 24 conserv + 52 non-conserv
  - Most Gp1 IgE epitopes: Non-homologous regions

- **Group 5 Grass Allergens**
  - Found in temperate but not subtropical sp.
    - Perennial rye Lol p5, Timothy Phl p5, Velvet Hol l5
  - rHol l5 epitope map: Overlapping peptides
    - Schramm et al, Clin Exp Allergy 2001; 31: 331-341
  - Fragments: May not ID discontinuous epitopes
    - Obs. in studies of Bet v 1, Der p 1 and Der p 2 structures
      - Short fragments (30 aa): Continuous sequ, low avidity
      - Long fragments: Discont. sequ, higher avidity
  - rHol l5 IgE epitopes: 4 continuous + 5 discontinuous

**Velvetgrass rHol l5 F16 Reactions**

**IgE Competitive Inhibition Assays**

- **ELISA inhibition**
  - Quantitative/adsorbed native Ag extracts
    - Reveals degrees of compositional similarity
    - Estimates rel. potencies of test & reference Ags
- **SDS-PAGE immunoblot inhibition**
  - Qualitative/semi-quantitative w/ denatured Ags
    - Identifies common, partial & unique structures
      - Conformational epitopes (3°) may be impaired
**ELISA / Blot Inhibition Format**

Solid-phase Allergen Serum Ab + Solution-phase Ag αIgE-AP Conjugate Substrate

**ELISA Inhibition Patterns**

- Log Dilution

% Inhibition

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**Grass-Pos IgE Immunoblot Profiles**

Greer Subject # G001

Jo Ba Ti Sv Rt Pr Or Mf Kb Be MW Stds kDa
97.4
66.2
45
31
21.5
14.4

**Blot Inhibitions Dose-Response**

Pt G060 Immob: Tim Inhib: P. rye

µg/mL 0 .01 1 10 100 N Gold Stds kDa
97.4
66.2
45
31
21.5
14.4

**Blot Inhibitions 100 µg/mL protein**

Pt G060 Immob: Tim Inhib: various

kDa Stds Gold P Be Mf Or Pr Rt Ti Be J N
97.4
66.2
45
31
21.5
14.4

**Blot Inhibitions 100 µg/mL protein**

Pt G060 Immob: S. vernal Inhib: various

kDa Stds Gold P Be Mf Or Pr Rt Ti Be J N
97.4
66.2
45
31
21.5
14.4
**Dust Mite Cross-Reactivities**

- Near-complete betw Dermatophagoides spp.
  - Shared (D. farinae, D. pter) + unique (mostly D. pter)
- Moderate w/ related dust/ storage mites
  - *Df/Dp/Euroglyphus magnei* family Pyroglyphideae
  - *Acarus siro* family Acaridae
  - *Tyrophagus putrescentiae* family Acaridae
- Limited with other dust/ storage mites
  - *Blomia tropicalis* family Echimyopidideae
  - *Lepidoglyphus destructor* family Glycyphagideae

**Prominent Dust Mite Allergens**

<table>
<thead>
<tr>
<th>Group</th>
<th>Activity or function</th>
<th>Mol wt (kd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cysteine protease</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>Reproductive/molting protein</td>
<td>14-15</td>
</tr>
<tr>
<td>3</td>
<td>Trypsin-like serine protease</td>
<td>29-30</td>
</tr>
<tr>
<td>4</td>
<td>Amylase</td>
<td>56-60</td>
</tr>
<tr>
<td>6</td>
<td>Chymotrypsin-like serine protease</td>
<td>25</td>
</tr>
<tr>
<td>8</td>
<td>Glutathione-S-transferase</td>
<td>26</td>
</tr>
<tr>
<td>9</td>
<td>Collagenolytic serine protease</td>
<td>24-28</td>
</tr>
<tr>
<td>10</td>
<td>Tropomyosin</td>
<td>33</td>
</tr>
</tbody>
</table>

**Prevalence of Positive IgE Reactions**

![Bar graph showing prevalence of positive IgE reactions for different mite allergens.](image)

**Dust Mite Cross-Reactivities**

- Conserved structures within each Ag group
  - Same or different epitopes for IgE & IgG

**Der 2 Isoallergens**

- Lys100 → Arg (conserv), → Thr/Glu (non-conserv)
  - Smith & Chapman *Clin Rev Allergy Imm* 1997; 27: 593-599
- MAb binding to Der 2 Arg100, Glu100 vs. Lys100
  - mAb 15E11 Arg100: slight
  - Glu100: slight
  - mAb 13A4 Arg100: total
  - Glu100: total
- Ab specificity critical for Der 2 Ag quantitation
  - 7A1 used as probe Ab in commercial Der 2 ELISA kits
**Insect Cross-Reactivities**

- Limited data, very few studies conducted
- Species-specific reactions are most common
- CRs observed among cockroach genera
  - *American* (*Periplaneta*), *German* (*Blatella*), *Oriental* (*Blatta*)
  - *Per a 1 / Bla g 1*: 70% aa identity
  - *Per a 7* (*Tropomyosin*): 80% homology w/ *inverts*
  - Shrimp, mites, crustacea/mollusks
  - 45% homology w/ *verts*
  - Beef, chicken, pork, lamb

**Animal Cross-Reactivities**

- Animal serum albumins exhibit high CRs
  - Cat, dog, human, horse, ...
- Major cat & dog allergens may CR in some pts
- Major mouse and rat urinary allergens may also CR in some pts
  - *Mus m 1 / Rat n 1*: Lipocalins, 65% sequence homology

**Mold Cross-Reactivities**

- Partial-complete within a genus: *Aspergillus*
- Partial between some genera:
  - *Alternaria, Stemphylium, Cladosporium, Curvularia, Stachybotrys*:
    - *Alt a 10 / Cla h 3*: Alddehyde dehydrogenases
    - *Alt a 11 / Cla h 6*: Enolase
    - *Alt a 6 / Cla h 4*: Acidic ribosomal protein P2
    - *Alt a 7 / Cla h 5*: *S. cerevisiae* protein YCP4
- None-partial within a genus: *Penicillium*

**Food Cross-Reactivities**

<table>
<thead>
<tr>
<th>Group</th>
<th>In vitro/sp IgE</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>Common</td>
<td>Common</td>
</tr>
<tr>
<td>Legume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish</td>
<td>Common</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Crustacean/Mollusk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tree nut</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg-chicken</td>
<td>Occasional</td>
<td>Rare</td>
</tr>
<tr>
<td>Milk-beef</td>
<td>Occasional</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Tree nut-pollen</td>
<td>Occasional</td>
<td>Occasional</td>
</tr>
</tbody>
</table>

**Food Cross-Reactivities**

- Proteins from distant/unrelated taxonomies with similar functions
- Syndromes:
  - Pollen-food
  - Birch/mugwort/fruit
  - Latex-food
  - Banana/avoc/chestnut
- Shared allergens:
  - Plant defense/pathogenesis-related proteins
  - Profilins
  - CCDs
  - Cross-reactive carbohydrate determinants

**Oral Allergy Syndrome (OAS)**

- Pollen Ag sensitization linked to food allergy
  - 1st reported with birch pollen & apples ~ 50 years ago
- Pollens and foods share homologous allergens
  - Taxonomically related in some (but not all) cases
  - Sensitizations to pollens precede reactions to foods
  - Pollens: Birch, mugwort, hazelnut
  - Foods: Apple, celery, carrot
- *Birch-celery*:
  - *Bet v 1 (PR10)*: 46-60 kD
  - *Bet v 2 (profilin)*: 46-60 kD
**Pathogenesis-Related (PR) Proteins**

- **Families**
  - **Conserved, stable Ag structures**
  - **PR2**
    - 1-3 glucanases
    - Latex Hev b 2
    - Barley, wheat, soybean, rice, mustard, cucumber
  - **PR3**
    - Endochitinases
    - Latex Hev b 6.02 (N term)
    - Banana, avocado, chestnut, kiwi, peach, rice, strawberry, citrus, potato, wheat, turnip
  - **PR4**
    - Endochitinases
    - Latex Hev b 6.03 (C term)
    - Potato, soybean, tobacco
  - **PR5**
    - Antifungal
    - Mt cedar, cherry, apple, pepper
  - **PR8**
    - Chitinase
    - Latex hevamine, cucumber
  - **PR10**
    - Bet v 1 homologs
  - **PR14**
    - LTPs
    - Lipid transfer proteins

- **PR10 Bet v 1 Family**
  - **Defense against bacterial & fungal pathogens**
  - Major Ags shared by many fruits, vegetables, nuts, pollens
  - **Homologs**
    - 17-22 kd, 154-160 aa, common 2° struc
    - Birch, alder, hornbeam, hazelnut, chestnut, oak pollens
    - Apple, pear, cherry, soybean, celery, carrot foods
  - **Isoallergens**
    - Birch Bet v 1, Apple Mal d 1
    - Single aa diffs sufficient to destroy CRs
  - **CRs associated w/ higher % of skin test+ and symptom+ pts vs. Bet v 2 family (profilins)**

- **Bet v 1 Isoallergens**
  - **7 isoforms (1-5aa diffs) ID by IEF immunoblots**
    - Akkerdaas et al Allergy 1995; 50: 215-220
  - **Hi gE/MIgG Ab reactivities to Bet v 1 isoforms**
    | pI  | IgE JH | MAb 5H8 | MAb 7F7 | MAb 9C11 | MAb 11E12 | MAb 3C4 |
    |-----|--------|---------|---------|---------|----------|---------|
    | 5.0 | +      | -       | -       | +       | +        | -       |
    | 4.8 | +      | -       | -       | +       | +        | -       |
    | 4.6 | +      | +       | +       | +       | +        | -       |
    | 4.4 | +      | +       | +       | +       | +        | -       |
    | 4.2 | +      | +       | +       | +       | +        | -       |

- **PR14 LTP Family**
  - **Inhibit bacterial and fungal pathogens**
  - Independent sensitizations: resp (pollen) and oral (food)
  - **Homologs**
    - 9-10 kd, 91-95 aa, phys/chem resistant
    - Mugwort, chestnut, sycamore, parietaria pollens
    - Apple, peach, plum, apricot, soybean, corn, wheat foods
  - **Pts w/ no birch or grass pollen sensitivities**
    - ID by ST w/ plum: high [LTP], very low [Bet v 1] & [Bet v 2]
  - **Anaphylaxis: 2X higher for PR14+ vs. PR10+ pts**
    - LTPs concentrated in outer layer (peel), much less in pulp
    - Severe reactions to some fruits attributed to LTPs alone

**Profilins  Bet v 2 Family**

- Cytoskeletal, actin-associated, fertilization sensitizations via pollens, CRs with fruits and vegetables
- Homologs 12-15 kd, 30-80% sequence homology
  - Birch, alder, hornbeam, hazelnut pollens
  - Apple, pear, peach, cherry, apricot, celery, carrot, potato, tomato, pumpkin foods and spices
- Minor (20%) panallergen CRs w/ Latex Hev b 8
- CRs associated w/ higher % of CAP/ RAST+ pts vs. Bet v 1 family (PR10)

**Cross-Reactive Carbohydrate Determinants (CCDs)**

- Non-mammalian, immunogenic, stable, rigid
  - Non-mammalian, immunogenic, stable, rigid
    - Man 1 → 6
    - Man 1 → 4 + GlcNAc 1 → 4 + GlcNAc → Asn
    - Xyl 1 → 2
    - Fuc 1 → 3
- Found in wide variety of allergenic materials
  - Pollens and foods + insect venoms, shellfish
- High IgE binding, very low clinical relevance

**Latex-Food Cross-Reactivities**

<table>
<thead>
<tr>
<th>Latex protein</th>
<th>CR group or food</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hev b 2</td>
<td>PR2  β1-3 glucanase</td>
</tr>
<tr>
<td>Hev b 3</td>
<td>Red kidney bean</td>
</tr>
<tr>
<td>Hev b 5</td>
<td>Kiwi</td>
</tr>
<tr>
<td>Hev b 6</td>
<td>PR3/4 Endochitinases</td>
</tr>
<tr>
<td>Hev b 7</td>
<td>Potato</td>
</tr>
<tr>
<td>Hev b 8</td>
<td>Bet v 2 family  Profilins</td>
</tr>
<tr>
<td>Hev b 9</td>
<td>Enolases  Alt/Clad enolase</td>
</tr>
<tr>
<td>Hev b 10</td>
<td>SODs  Aspergillus Mn²⁺ SOD</td>
</tr>
</tbody>
</table>

**Conclusions**

- Knowledge of CR patterns/mechanisms continues to expand at a rapid pace
- Clinical/biochemical tests (+ taxonomy): Positive ID of many important CR allergens
- Incorporation in Dx/Rx practices effective but many CR relationships remain obscure
- Collaborative studies essential to identify, understand & manage clinically-relevant CRs

**Selected References**

- Weber RW

**Q&A**

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