Allergy Diagnosis 2013

Between Scientific Progress and Unproven Methods

Workshop 5703

Antibody-Based Tests

IgE in Immediate-Type Hypersensitivity to IgG4 Antibodies to Foods

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Workshop 5703
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Disclosures:

- Research projects with Thermofisher Scientific, Siemens Healthcare
- Advisory Board of Hycor Biomedical

Objectives

- IgE: Examine scientific progress in antibody based tests to assess immediate-type hypersensitivity from 1967 to present
- IgG4: Discuss origin of food-specific IgG4 test dogma in diagnosis and evidence they do NOT provide clinically useful information
IgE in Immediate-Type Hypersensitivity

Diagnostic Allergy Laboratory Assessment of Immediate-Type Hypersensitivity

- Allergen-specific IgE (~200 allergen specificities)
  - Aeroallergens: Pollens (weeds, grasses, trees), animal epidermals, dust mites, molds
  - Ingested Allergens: Foods
  - Injected Allergens: Venoms, Drugs
- Total Serum IgE (anti-IgE; ABPA)
- Multi-allergen atopic screen for IgE antibody
- Mast Cell Tryptase
Radioallergosorbent Test (RAST): First Generation

Allergen bound to paper disc

All antibody isotypes bind: Ig of A,M,G,E class

Bound IgE detected with polyclonal I\(^{125}\) Anti-IgE

Results reported as log-related classes or arbitrary units by interpolation of heterologous IgE anti-birch pollen curve

IgE was identified in 1967. The RAST 1st on the market in 1974, considerable variability & questionable; RAST is no longer appropriate


Current Single-plex IgE Antibody Autoanalyzers

HyTec-288:
Hycor Biomedical Inc.

ImmunoCAP (250, 1000):
Thermofisher Scientific Inc.
(formally Phadia/Pharmacia)

Immulite 2000/2500:
Siemens Medical Solutions Diagnostics
Current IgE Antibody Single-plex Autoanalyzers

Basic reagents and chemistries are similar

- Antibody binds to the allergen immobilized on a solid phase
- Following buffer washes, enzyme anti-IgE detects bound IgE
- Following buffer washes, substrate produces a measured response
- All assays display comparable analytical sensitivities to 0.1 kUa/L
- All assays use allergens from extracts and some components

<table>
<thead>
<tr>
<th>Name</th>
<th>Company*</th>
<th>Solid Phase Type</th>
<th>Enzyme-labeled Detection Antibody</th>
<th>Substrate**</th>
<th>Calibration System</th>
<th>Analytical Sensitivity***</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTS-E28</td>
<td>Hyser-Aglon</td>
<td>Paper disc</td>
<td>Alkaline phosphatase labeled anti-IgE</td>
<td>p-nitrophenyl phosphate</td>
<td>Total IgE system</td>
<td>0.1 kUa/L</td>
</tr>
<tr>
<td>ImmunoCAP</td>
<td>Phadia</td>
<td>Cellulose Bead</td>
<td>β-galactosidase labeled anti-IgE</td>
<td>4-methylumbelliferyl-β-D-galactoside</td>
<td>Total IgE system</td>
<td>0.1 kUa/L</td>
</tr>
<tr>
<td>Immulite</td>
<td>Siemens</td>
<td>Biotinylated allergen &amp; anti-IgE particle</td>
<td>Alkaline phosphatase labeled anti-IgE</td>
<td>4-methylazoxystilbene-2,2'-diamine</td>
<td>Total IgE system</td>
<td>0.1 kUa/L</td>
</tr>
</tbody>
</table>

*Alkaline phosphatase labeled anti-IgE
**p-nitrophenyl phosphate
***4-methylumbelliferyl-β-D-galactoside

Progress in IgE Antibody Serology

- Clinical assays: manual to singleplex autoanalyzers shortens assay turnaround, enhances reproducibility within and between laboratories, and allows calibration against common international IgE standard
- Evolution from exclusive allergen extract-based assays to increased use of individual allergenic components (native or recombinant)
- Increased use of multiplexed IgE antibody assays using a component-allergen chip based system
ImmunoCAP Multi-plexed ISAC Chip

Strengths and Weaknesses of Allergen Component-based Analysis

**Strengths:**
- Identifies IgE antibodies to a specific proteins predictive of clinical reactivity.
- Can detect cross-reactivity among different allergen sources especially among the pollens and foods
- Identifies sensitivity patterns with small quantities of serum

**Weaknesses:**
- Semi-quantitative measurement
- May miss detecting IgE antibodies to some clinically relevant allergens which are not present in the assay because they have not yet been identified or clinically characterized.
IgG4 Antibodies to Foods


**Monoclonal antibodies to immunoglobulin G<sub>4</sub> induce histamine release from human basophils in vitro**

*J ALLERGY CLIN IMMUNOL 70:399, 1982*

Diana L. Fagan, B.S., Clive A. Slaughter, Ph.D., J. Donald Capra, M.D., and Timothy J. Sullivan, M.D. Dallas, Tex.

**Methods**

Human IgE, IgG1-4, IgM, IgA monoclonal antibodies prepared
Isolated peripheral blood leukocytes: EDTA-Dextran T500 + Ca
2 million leukocytes + MAb +/- goat anti-mouse IgG: 37C-45 min
Measure histamine content of supernatants
### TABLE III. Release of histamine from normal human peripheral blood leukocytes induced by anti-IgG4

<table>
<thead>
<tr>
<th>Subject</th>
<th>180 µg/ml</th>
<th>18.5 µg/ml</th>
<th>Second incubation—anti-IgG4 (168 µg/ml) + goat anti-mouse IgG (35 µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J. Z.</td>
<td>6.1 ± 0.6</td>
<td>6.2 ± 1.1</td>
<td>14.5 ± 4.0</td>
</tr>
<tr>
<td>J. Z</td>
<td>10.9 ± 6.1</td>
<td>58.6 ± 4.7</td>
<td>22.3 ± 3.3</td>
</tr>
<tr>
<td>J. Z.</td>
<td>13.7 ± 3.4</td>
<td>5.3 ± 2.5</td>
<td>0.4 ± 7.5</td>
</tr>
<tr>
<td>T. R.</td>
<td>13.2 ± 0.2</td>
<td>12.3 ± 0.5</td>
<td>14.1 ± 0.1</td>
</tr>
<tr>
<td>T. R.</td>
<td>20.9 ± 0.1</td>
<td>19.8 ± 2.3</td>
<td>13.2 ± 0.8</td>
</tr>
<tr>
<td>E. H.</td>
<td>30.5 ± 4.7</td>
<td>13.8 ± 0.7</td>
<td>14.4 ± 4.8</td>
</tr>
<tr>
<td>M. K</td>
<td>20.3 ± 0.2</td>
<td>1.7 ± 0.1</td>
<td>—</td>
</tr>
</tbody>
</table>

### TABLE IV. Release of histamine from normal human peripheral blood leukocytes induced by anti-IgG, anti-IgG4, anti-IgG2, anti-IgM, and anti-IgA

<table>
<thead>
<tr>
<th>Addition</th>
<th>No. of experiments</th>
<th>Concentration of anti-immunoglobulin*</th>
<th>Anti-Ig (200 µg/ml) + goat anti-mouse IgG (35 µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-IgG4</td>
<td>3</td>
<td>-0.3 ± 1.0</td>
<td>1.7 ± 1.9</td>
</tr>
<tr>
<td>Anti-IgG</td>
<td>3</td>
<td>1.8 ± 2.2</td>
<td>1.5 ± 1.5</td>
</tr>
<tr>
<td>Anti-IgG2</td>
<td>6</td>
<td>-1.4 ± 2.1</td>
<td>0.8 ± 0.8</td>
</tr>
<tr>
<td>Anti-IgM</td>
<td>3</td>
<td>-1.0 ± 1.8</td>
<td>-0.3 ± 0.7</td>
</tr>
<tr>
<td>Anti-IgA4</td>
<td>5</td>
<td>-9.1 ± 0.7</td>
<td>-0.3 ± 0.7</td>
</tr>
</tbody>
</table>

### Conclusions

- Monoclonal anti-HuIgG4 induces basophil histamine release from 4 of 14 subjects (29%).
- Passive sensitization with IgG4 myeloma was not successful
- Functionally relevant IgG4 could be on the surface of basophils or other stimulating cells
ANTI-HUMAN IgG CAUSES BASOPHIL HISTAMINE RELEASE BY ACTING ON IgG-IgE COMPLEXES BOUND TO IgE RECEPTORS

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Methods

IJUIS document monoclonal anti-human IgG subclass 1-4 Serum & peripheral blood leukocytes (EDTA 6% Dextran 70) from 24 atopics and 13 non-allergics Mab + leukocytes – 45 min @ 37C Histamine release was measured in supernatants

Figure 1. Basophil histamine release from washed cells from two atopic donors (MT, upper panel and JG, lower panel) after incubation with 0.004 to 300 µg/ml of polyclonal anti-IgE or anti-human IgG1-4 mAb. Maximal histamine release with all IgG1-4-specific mAb was achieved with 50 to 300 µg/ml which is 1000-fold higher than the concentration of polyclonal anti-IgE required for optimal release.
Figure 2. The frequency and magnitude of basophil histamine release from basophils obtained from 12 nonatopic (N) and 25 atopic (A) donors. Atopic donors who released to anti-IgG are denoted by A' (atopic releaser) whereas those who did not are denoted by A'' (atopic non-releaser). No basophil histamine release to anti-IgG (<10%) was observed with cells from nonatopic donors. All but two nonatopic donor cells released to anti-IgG. Of the 25 atopic donor cells, 18 released histamine to at least one human IgG1-4 mAb. The individual patterns of release to the individual anti-IgG mAb are presented in Table II.

Figure 6. Inhibition by IgE myeloma of passive sensitization of lactic acid-treated basophils using a serum from a ragweed-sensitive donor (TK) and a serum from an IgG-responding donor (EH). The cells were incubated for 90 min at 37°C in serum without or with increasing concentrations of IgE myeloma, washed, and then exposed to ragweed Ag E or anti-IgG. Control histamine release to ragweed Ag E was 21% and that to mAb anti-IgG3 (HP6050) was 26%. Similar results were obtained in three additional experiments.
**TABLE IV**

*Passive sensitization of leukocytes with absorbed serum*

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>% Histamine Release</th>
<th>A*</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>7/6</td>
<td>6/7</td>
<td>7/8</td>
<td>8/5</td>
<td></td>
</tr>
<tr>
<td>mAb 6050 (anti-IgG3 100 μg/ml)</td>
<td>0/0</td>
<td>0/0</td>
<td>0/1</td>
<td>35/44</td>
<td></td>
</tr>
<tr>
<td>mAb 6047 (anti-IgG3 100 μg/ml)</td>
<td>0/0</td>
<td>0/1</td>
<td>0/1</td>
<td>25/57</td>
<td></td>
</tr>
</tbody>
</table>

* Experimental conditions: A, not acid-treated, not sensitized; B, acid-treated, not sensitized; C, acid-treated, sensitized, IgE removed; D, acid-treated, sensitized, IgE not removed.

* Results for two leukocyte donors.

Anti-human IgG induced histamine release from basophils is a result of human IgG antibody that is complexed with IgE and attached to the surface of the basophil from atopic donors through the high affinity IgE receptor.

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Testing for IgG4 against foods is not recommended as a diagnostic tool: EAACI Task Force Report* Allergy 2008: 63: 783–796

IgG4 anti-food (A) 100 unselected 12-16 yr school children; (B) 264 - 1 yr olds  [Eysink et al., Clin Exp. Allergy 21:99,1999] milk, egg white, orange, banana, pork, potato, soy/peanut, wheat
CONCLUSIONS

* IgG4 anti-food levels do not correlate with IgE anti-food levels in same individuals

* Food specific IgG4 antibodies indicate repetitive exposure; possibly immunological tolerance linked to T-regulatory activity (e.g. hyper-stung bee keepers; clinically successful immunotherapy)

* Food specific IgG4 does not indicate food allergy or intolerance

* Testing for IgG4 anti-food is irrelevant for a food allergy work-up