Non-IgE mediated mechanisms of allergy (genetics, molecular pathways, diagnostic models).

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Disclosure

- PI in DBV SMILEE trial (protect time to conduct research)
- Coinvestigator in MILES, ARA101, PALISADES (protect time to conduct research)
- FARE Co-director of the FARE CHOP Center (protect time to conduct research)
- Funding from APFED/ART trust – research funding
- AAAAI-BCI secretary -volunteer
- EAACI-EoE IG-Board-volunteer
<table>
<thead>
<tr>
<th>IgE mediated</th>
<th>Mixed</th>
<th>Not IgE mediated</th>
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<tbody>
<tr>
<td>Urticaria</td>
<td>Atopic dermatitis</td>
<td>Food Protein-Induced Enterocolitis</td>
</tr>
<tr>
<td>Angioedema</td>
<td></td>
<td>Eosinophilic Esophagitis</td>
</tr>
<tr>
<td>Anaphylaxis</td>
<td></td>
<td>Eosinophilic Gastritis</td>
</tr>
<tr>
<td>Oral Allergy Syndrome</td>
<td></td>
<td>Eosinophilic colitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eosinophilic gastroenteritis</td>
</tr>
</tbody>
</table>
Non-IgE mediated food allergy

Eosinophilic Esophagitis (DELAYED REACTION-CHRONIC INFLAMMATION)

FPIES (DELAYED REACTION-ACUTE INFLAMMATION)

Chemokine cytokines

Immediate Late Phase

Minutes Hours

0 30 60 6 8 10 12

IgG IgA

B cell T cell

Epithelial cell

EOS

low affinity

Masty

APC
Center for Pediatric Eosinophilic Disorders (CPED)

- The largest clinical center in the world with over 1800 patients with EoE

- CHOP Accomplishments
  - Identified the Genetic risk (TSLP, EMSY, CAPN14) factor for EoE
  - Wrote the critical manuscript defining natural history
  - Developed a new clinical test for food sensitivity in EoE
  - Characterized efficacy of two treatment interventions
  - Orchestrated the first multicenter consortium for EoE
  - First clinical trial in Milk desensitization in the world (SMILEE)
Clinicopathologic diagnosis

-Presence of clinical symptoms related to esophageal dysfunction
  • Dysphagia, vomiting, abdominal pain, heartburn, feeding difficulty, etc.

-Isolated esophageal eosinophilia
  • 15 or more eosinophils per hpf

-Histology of remainder of Gl tract normal

-Exclusion of other Gl disorders
  • Absence of pathologic GERD

-Lack of response to PPI therapy (1-2 mg/kg for 8 weeks) or normal pH probe

-Infection, Crohn's disease, hypereosinophilic syndrome

Furuta, et al; Gastroenterology 2007; 133:1342.
Symptom Progression in EoE

- Feeding Disorder/Failure to thrive: 13%
- GERD/vomiting: 50%
- Abdominal Pain: 50%
- Dysphagia: 30% (Pediatric), 97% (Adults)
- Food Impaction: 13% (Pediatric), 51% (Adult)
- Esophageal Stricture: 10% (Pediatric), 37% (Adult)

Age
Quality of Life EoE is reduced and improved with treatment

1) subject age, EoE symptom burden, atopic comorbidities, and treatment type were associated with baseline quality of life ratings of child and family impact.
2) EoE symptom severity scores decreased during the study, although number of symptoms did not.
3) Symptom burden scores were consistently correlated with Quality of life scores at baseline and follow-up time points.
4) HRQoL improved during the course of evaluation and treatment, with positive changes being strongest for patients with lower symptom severity at BL.

Klinnert, JPGN, 2014
Eosinophilic Esophagitis
Long term follow up @ CHOP

- 1995-2006 (512 patients with EoE)
  - Follow up to 14.2 yrs
    - Average of 2.4 yrs with a total of 1782 biopsies
  - No cases of EoE becoming Eosinophilic gastroenteritis
  - 24 patients refused therapy or lost to F/U
    - Years since 1st visit – 6.2±3.6
    - # eosinphils 1st EGD – 35.4±24.8
    - # eosinophils recent EGD – 39.1±27.9
    - 20/24 initially presented with GERD symptoms
      - All returned with symptoms of dysphagia

Spergel et al. JPGN 2009
## Atopy and EoE

<table>
<thead>
<tr>
<th>Author/Population</th>
<th>N</th>
<th>Asthma</th>
<th>Allergic Rhinitis</th>
<th>Atopic Dermatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopy in the general Population</td>
<td></td>
<td>8.5%</td>
<td>25%</td>
<td>10%</td>
</tr>
<tr>
<td>Spergel et al Children</td>
<td>620</td>
<td>50%</td>
<td>61%</td>
<td>21%</td>
</tr>
<tr>
<td>Ass’aa, et al Children</td>
<td>89</td>
<td>39%</td>
<td>30%</td>
<td>19%</td>
</tr>
<tr>
<td>Sugnanam et al Children</td>
<td>45</td>
<td>66%</td>
<td>93%</td>
<td>55%</td>
</tr>
<tr>
<td>Guajardo et al Children and Adults</td>
<td>39</td>
<td>38%</td>
<td>64%</td>
<td>26%</td>
</tr>
</tbody>
</table>
### Who are the patients with EoE?

<table>
<thead>
<tr>
<th>Adjusted OR</th>
<th>Caucasian</th>
<th>Male</th>
<th>MHV&lt; $100,000</th>
<th>MHHI &lt; $100,000</th>
<th>College</th>
<th>Suburban</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EE-GI</strong></td>
<td>1.90 (1.26-2.85)</td>
<td>3.49 (2.52-4.83)</td>
<td>0.80 (0.45-1.40)</td>
<td>0.88 (0.53-1.46)</td>
<td>1.05 (0.91-1.22)</td>
<td>0.82 (0.46-1.45)</td>
</tr>
<tr>
<td><strong>EE-Allergy</strong></td>
<td>1.98 (1.32-2.96)</td>
<td>1.52 (1.11-2.08)</td>
<td>1.06 (0.60-1.87)</td>
<td>0.78 (0.47-1.31)</td>
<td>0.96 (0.83-1.11)</td>
<td>2.08 (1.22-3.54)</td>
</tr>
</tbody>
</table>

**Sibling risk** = The ratio of risk of disease manifestation, given that one's sibling is affected, as compared with the disease prevalence in the general population.

Asthma-2

EOE-80

Franciosi et al, Clin Gastro Hepatol 2009
Triggers

- Environmental Allergens
- Infections
- Foods
Food Allergy and EoE: lesson learned from elemental diet

- Kelly and Sampson
  - 10 patients (5 yr, range: 8 mo-12.5 yr)
  - Endoscopy pre- & post-trial

Kelly et al. Gastroenterology 1995

Lucendo, 2015
How to predict the food implicated in food allergy driven EoE

- History not accurate
  - Reactions may be delayed several days
  - Reactions may persist several days
  - More than one food can cause reaction

- Percutaneous Testing for most common foods
  - Strong NPVs (Negative predictive value) (NOT FOR MILK)
  - Low PPVs (Positive predictive values) 50-85% depending for which food

- IgE Microarray (CRD)-based dietary treatment was not effective in adult patients with EoE
  - Missed sensitizations
  - Limited relevance of IgE in the pathophysiology of EoE

Kelly, et al, Gastroenterology 1995
Liacouras et al Clin Gastroenterology and Hepatology 2005
Spergel JACI 2007
Which Foods?

- Guess, Empiric (6 food elimination diet)
  - Remove the most common foods (milk, soy, egg, wheat, seafood, peanuts/treenuts)

- Test (targeted elimination diet)
  - Prick Skin test/In vitro specific IgE
  - Atopy Patch test
  - Accurate diet history—what patients eat
  - Patients advised to remove positive foods for the diet
  - Patients with multiple food allergies placed on elemental diet

Kelly, et al, Gastroenterology 1995
Liacouras et al Clin Gastroenterology and Hepatology 2005
Spergel JACI 2007
Six Food Elimination Diet (SFED) Adults

- Food Reintroduction
  - most common food triggers were wheat (60%), milk (50%), soy (10%), nuts (10%), egg (5%), seafood (0)
  - Three patients had more than one food trigger
  - SPT accurately predicted only 13% of causal agents, and 67% of patients who had a food trigger identified by the reintroduction process had a negative SPT to all foods

Gonsalves et al. Gastroenterology 2012
Most Common Foods in EoE

All pts had > 20 eos/hpf on GERD and AR medication and had
-Removal of a single food leading to normal esophageal biopsy (0 eosinophils/HPF).
-Addition of a single food leading to increased esophageal eosinophils on biopsy after a previously normal biopsy.
319 Children had definitive causative food (out of 941 patients examined)

<table>
<thead>
<tr>
<th>Food</th>
<th>No. of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>78</td>
</tr>
<tr>
<td>Milk, meats*</td>
<td>24</td>
</tr>
<tr>
<td>Milk, egg, wheat, soy</td>
<td>20</td>
</tr>
<tr>
<td>Milk, soy</td>
<td>15</td>
</tr>
<tr>
<td>Grains*</td>
<td>13</td>
</tr>
<tr>
<td>Milk, egg, wheat, meats</td>
<td>11</td>
</tr>
<tr>
<td>Egg, wheat</td>
<td>10</td>
</tr>
<tr>
<td>Milk, egg</td>
<td>8</td>
</tr>
<tr>
<td>Milk, egg, wheat</td>
<td>8</td>
</tr>
<tr>
<td>Egg</td>
<td>8</td>
</tr>
<tr>
<td>Soy</td>
<td>7</td>
</tr>
<tr>
<td>Wheat</td>
<td>5</td>
</tr>
</tbody>
</table>

Younger children sensitive to more foods

Spergel et al JACI 2009
Some patient with milk induced EoE are able to tolerate baked milk

Leung J, JACI 2013
It is well known that cooking and/or processing can
- denature conformational epitopes, making them no longer recognizable by the epitope-specific IgE.
- strengthen certain protein bonds or create neoepitopes, such as when amino acids react with aldehyde or ketone groups on sugars (glycation) in enzymatic browning or roasting known as the Maillard reaction. (roasting peanuts or cooking shellfish) Peanut protein component Ara h2 forms aggregates during this reaction that are harder to digest and more easily recognized by epitope-specific IgE.
- The predominant protein in Egg White, ovalbumin (OVA), is a conformational epitope and heat labile, whereas the other major allergen, OM, is a sequential epitope and heat resistant, making OM potentially more allergenic.
- The whey proteins in CM, such as alpha-lactalbumin and beta-lactoglobulin, contain conformational epitopes that are heat labile (significantly reduced after 20 minutes of boiling), whereas casein contains mostly sequential and heat-resistant epitopes.
• Heating is only one part of rendering baked milk and egg less allergenic. Interactions with proteins, fats, or sugar in a food matrix, such as wheat, are equally important.
• This is why the simple act of boiling cow’s milk may not be enough to decrease allergenicity to a degree comparable with a baked product.
• The food matrix may help to reduce exposure of the specific proteins to the immune system.
• For example, the beta-lactoglobulin fractions of whey form disulfide bonds with the other proteins in the food matrix, making them less recognizable by specific IgE.
• Ovomucoid polymerizes with proteins in the food matrix, such as gluten, to form large insoluble aggregates, making it less recognizable by epitope-specific IgE and potentially less allergenic.
There is clearly some uncertainty about the necessity of avoiding rye and barley in addition to wheat in elimination diets in adults and children with EoE. Published and unpublished data from our centers and others (including studies of patients with EoE and gluten-triggered celiac disease) are too limited to speculate whether total gluten elimination (wheat, barley, and rye) might be meaningfully more efficacious than elimination of only wheat in patients with EoE. Unless the theoretic risks of wheat, barley, and rye cross-reactivity/cross-contamination are confirmed with empiric evidence in patients with EoE, we advise against extending wheat elimination to include the exclusion of other gluten-containing grains.
Pharmacologic Therapy

**Systemic Steroids** – effective at improving symptoms and histology of EoE in 95% of pts
- Upon discontinuation, 90% had recurrence of symptoms
- *(Long term use) Side effects*: bone abnormalities, poor growth, adrenal suppression
- May be needed short term for extreme cases

**Topical/swallowed Steroids** – less toxic to pt while still 50-85% effective
- A mainstay of EoE treatment in adults and children.
- Upon discontinuation almost all patients have a recurrence of symptoms
- Often, large doses needed
- *Side effects*: esophageal candidiasis
- Potential: growth impairment and osteoporosis

Liacouras *et al.* Clin Gastroenterol Hepatol 2005
Furuta *et al.* Gastroenterology 2007
Cianferoni a *et al.* 2015
• Anti-IL-5 (mepolizumab) reduces Eosinophilic infiltration in humans but has no significant effects on dysphagia.
In a double blind placebo control trial, Omalizumab has no effect on eosinophil content or relative to placebo controls, symptoms.

EoE ≠ IgE Mediated Disease

Clyaton F 2014
## Cytokines in EoE pathogenesis

<table>
<thead>
<tr>
<th>Publication</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Straumann et al. JACI 2002</strong></td>
<td>Increased expression of IL-5, TNF-α was increased in epithelial cells</td>
</tr>
<tr>
<td><strong>Straumann et al. Inflamm Bowel Dis, 2004</strong></td>
<td>Eosinophils express “activation” markers: 60% express <strong>IL-13 and IL-4</strong>; 41% of intestinal eosinophils express IL-13 at baseline, circulating eosinophils do not express IL-4 or IL-13</td>
</tr>
<tr>
<td><strong>Gupta et al. Am J Gastro 2006</strong></td>
<td>No increase in cysLT</td>
</tr>
<tr>
<td><strong>Blanchard et al. J Clin Invest 2006</strong></td>
<td>50-fold increase in <strong>Eotaxin-3</strong> in biopsy</td>
</tr>
<tr>
<td><strong>Aceves et al. JACI 2007</strong></td>
<td>Esophageal remodeling with increased TGFβ, phospho-Smad2/3, VCAM</td>
</tr>
<tr>
<td><strong>Battacharya et al, Hum Pathol 2007</strong></td>
<td>Increased Eotaxin-3 in archived biopsies</td>
</tr>
<tr>
<td><strong>Blanchard C et al JACI 2011</strong></td>
<td>Increased <strong>IL-13, IL-4, IL-5 IL-15</strong> in esophageal bx of patients with active EoE</td>
</tr>
<tr>
<td><strong>Zhou H et al Gastroenterology 2010</strong></td>
<td>Eosinophialia in Esophageal bx correlates with IL-15 levels</td>
</tr>
<tr>
<td><strong>Rothenberg ME, Spergel JM, Nat Genet. 2010</strong></td>
<td>Increased TSLP in esophageal bx of patients with active EoE</td>
</tr>
</tbody>
</table>
How food can induce Eosinophilic-Th2 inflammation?

Food allergens, other triggers?

Eosinophilic-Th2 Inflammation
1) The detection of specific IgEs for food allergens, either by SPT or by specific sera IgE (sIgE), has not proven successful for the identification of causative foods in EoE.

2) Clinical trials and case series have shown that therapy with omalizumab is not effective in inducing remission of EoE.

3) Oral immunotherapy, which has been used successfully in IgE-mediated food allergy, is associated with an increased risk of developing EoE (e.g. in 2 to 10% of treated patients).

4) Children who outgrow IgE-mediated food allergy and therefore are able to reintroduce these foods in their diet can later develop EoE to the same food.

5) In experimental models in which food allergens are able to induce an EoE-like disease, mice with depleted IgE and devoid of mast cells still could develop esophageal inflammation and consequent food impaction similar to the wild-type mice.

Simon D, Cianferoni A et al Allergy 2016
EoE ≠ IgE Mediated Disease

Resolution of acute IgE-mediated allergy with development of eosinophilic esophagitis triggered by the same food

Showed this mechanism in about 30 patients
- Candidate Gene
- Genomic Wide Association Studies
Common variants at 5q22 associate with pediatric eosinophilic esophagitis

Marc E Rothenberg1,11, Jonathan M Spergel2,3,11, Joseph D Sherrill1,11, Kiran Annaiah4,11, Lisa J Martin5,11, Antonella Cianferoni1–3, Laura Gober2, Cecilia Kim4, Joseph Glessner4, Edward Frackelton4, Kelly Thomas4, Carine Blanchard1, Chris Liacouras3,6, Ritu Verma3,6, Seema Aceves7, Margaret H Collins8, Terri Brown-Whitehorn2,3, Phil E Putnam9, James P Franciosi9, Rosetta M Chiavacci4, Struan F A Grant3,4,10, J Pablo Abonia1, Patrick M A Sleiman4 & Hakon Hakonarson3,4,10

Table 1 Results for the most strongly associated SNPs at 5q22

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position</th>
<th>Minor allele</th>
<th>Discovery cases MAF</th>
<th>Discovery controls MAF</th>
<th>Discovery P trend</th>
<th>Discovery OR</th>
<th>Replication cases MAF</th>
<th>Replication controls MAF</th>
<th>Replication P trend</th>
<th>Replication OR</th>
<th>Fisher’s combined P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3806932</td>
<td>110,433,574</td>
<td>G</td>
<td>0.312</td>
<td>0.465</td>
<td>7.18 x 10^-6</td>
<td>0.54</td>
<td>0.377</td>
<td>0.452</td>
<td>0.008</td>
<td>0.73</td>
<td>3.19 x 10^-7</td>
</tr>
<tr>
<td>rs7723819</td>
<td>110,455,246</td>
<td>A</td>
<td>0.326</td>
<td>0.467</td>
<td>3.09 x 10^-7</td>
<td>0.55</td>
<td>0.382</td>
<td>0.464</td>
<td>0.004</td>
<td>0.71</td>
<td>7.67 x 10^-9</td>
</tr>
<tr>
<td>rs10051830</td>
<td>110,480,744</td>
<td>A</td>
<td>0.323</td>
<td>0.453</td>
<td>1.41 x 10^-6</td>
<td>0.58</td>
<td>0.371</td>
<td>0.454</td>
<td>0.003</td>
<td>0.71</td>
<td>2.37 x 10^-8</td>
</tr>
<tr>
<td>rs1043828</td>
<td>110,491,907</td>
<td>C</td>
<td>0.464</td>
<td>0.350</td>
<td>1.26 x 10^-5</td>
<td>1.61</td>
<td>0.418</td>
<td>0.346</td>
<td>0.010</td>
<td>1.36</td>
<td>5.99 x 10^-7</td>
</tr>
</tbody>
</table>

EE TSLP

$P<0.0001$

TSLP mRNA expression (normalized to GAPDH)
TSLP: Thymic stromal lymphopoietic

- IL-7–like cytokine
  - Expressed in thymus and epithelial cells
  - Potent inducer maturation of dendritic cells
  - Primes TH cells into TH2 cells
  - Induced by virus, bacterial, allergen

- TSLP is ↑ in lesional skin in AD and asthma.

Th2 antigen response

Dendritic cells

Allergen - Protein

TSLP

Th2-Cytokine

Th2

Th0

Treg

Th1

Th17
Eosinophilic gastrointestinal disease and peanut allergy are alternatively associated with IL-5\(^+\) and IL-5\(^-\) T\(_H\)2 responses

Calman Prussin, MD, Joohee Lee, MD, and Barbara Foster, MS  Bethesda, Md
Th2 cytokines and epithelial dysfunction
GWAS identifies four novel eosinophilic esophagitis loci

Patrick M.A. Sleiman\textsuperscript{1,2}, Mei-Lun Wang\textsuperscript{2,3}, Antonella Cianferoni\textsuperscript{2,4}, Seema Aceves\textsuperscript{5}, Nirmala Gonsalves\textsuperscript{6}, Kari Nadeau\textsuperscript{7}, Albert J. Bredenoord\textsuperscript{8}, Glenn T. Furuta\textsuperscript{9}, Jonathan M. Spergel\textsuperscript{2,4} & Hakon Hakonarson\textsuperscript{1,2}

Genome-wide association analysis of eosinophilic esophagitis provides insight into the tissue specificity of this allergic disease

Leah C Kottyan\textsuperscript{1,2,13}, Benjamin P Davis\textsuperscript{1,13}, Joseph D Sherrill\textsuperscript{1}, Kan Liu\textsuperscript{1}, Mark Rochman\textsuperscript{1}, Kenneth Kaufman\textsuperscript{1,2}, Matthew T Weirauch\textsuperscript{1,4}, Samuel Vaughn\textsuperscript{1}, Sara Lazaro\textsuperscript{1,13}, Andrew M Rupert\textsuperscript{1}, Mojtaba Kohram\textsuperscript{1}, Emily M Stucke\textsuperscript{1}, Katherine A Kemme\textsuperscript{1}, Albert Magnusen\textsuperscript{1,2}, Hua He\textsuperscript{3}, Phillip Dexheimer\textsuperscript{4}, Mirna Chehade\textsuperscript{6}, Robert A Wood\textsuperscript{4}, Robbie D Pesch\textsuperscript{5}, Brian P Vickery\textsuperscript{9}, David M Fleischer\textsuperscript{10}, Robert Lindbad\textsuperscript{11}, Hugh A Sampson\textsuperscript{8}, Vincent A Mukkada\textsuperscript{12}, Phil E Putnam\textsuperscript{11,12}, Pablo Abonia\textsuperscript{2}, Lisa J Martin\textsuperscript{5}, John B Harley\textsuperscript{12,14} & Marc E Rothenberg\textsuperscript{1,14}
EoE pathogenesis

1) Genetics
2) Epithelium
3) Th2 inflammation

Esophageal epithelium

- TSLP
- RANTES
- Eotaxin 3
- CAPN14
- EMSY

Mastcells
Basophils
DC
Cell. T CD4+
iNKT
Eosinophils

TH2 Cytokines

Cianferoni A, 2015
Viaskin Technology

- Proteins are loaded into central polyethylene membrane charged with electrostatic forces.
- Delivery system creates occlusive chamber on skin that generates moisture & releases proteins from membrane.
- Proteins are then absorbed through skin where they interact with epidermal immune cells.

Conclusions

- Eosinophils are part of the inflammatory cells typical of Th2 inflammation, but are not essential for pathogenesis.
- A dysfunctional epithelium in atopic genetically susceptible individuals induce local atopic inflammation by:
  - Secreting mediators that promote Th2 cytokines
  - Increase access to antigens
  - Inducing local sensitization to allergen such as foods
  - Promote chronic inflammation
FPIES: Food protein enterocolitis

FPIES is a non-IgE-mediated food allergy hallmarked by delayed onset of profuse, repetitive emesis, and lethargy that may be accompanied or followed by watery/bloody diarrhea

International Consensus Guidelines AAAAI.
A Nowak-Węgrzyn, et al (Submitted)
Clinical features of FPIES

- Vomiting (typically around 2 hours post ingestion)
- Diarrhea (typically 5 hours post ingestion)
- Lethargy
- Dehydration that may progress to:
  - Acidemia
  - Hypotension
  - Methemoglobinemia
- May also find elevated PMN/PLTs count
- Occasional hypoalbuminemia and FTT
Treatment of acute reaction

- Intravenous fluid boluses
- Ondasetron
- Steroids
- Supportive care
- Epinephrine traditionally does NOT help
Allergy testing for food specific IgE by either prick skin testing or serologic assessment is typically of little or no value in the diagnosis of FPIES, as FPIES is not an IgE-mediated process.

Atopy patch testing (APT) has also been evaluated as a possible means of identifying specific food sensitivities in patients with FPIES.

However, only two studies performed to date have evaluated APT, with conflicting results as to its diagnostic value in predicting challenge outcome.

International Consensus Guidelines AAAAI.
A Nowak- Węgrzyn, et al (Submitted)
On 462 children (1031 episodes) Most common foods were

- Grain = rice, oat, WHEAT very rare
- Meat = chicken, turkey
- Vegetables = sweet potato, peas, squash
- Fruit = Banana, apple

Ruffner et al JACI in practice 2014
Foods That Trigger FPIES
(Mount Sinai Data)

Caubet, et al.  JACI 2014; 134:382-9
FPIES: Foods

- On 462 children (1031 episodes) Most children were allergic to only 1-2 foods

Ruffner et al JACI in practice 2014
Humoral and cellular responses to casein in patients with food protein–induced enterocolitis to cow’s milk

<table>
<thead>
<tr>
<th>TABLE 1. Summary of pathologic findings in patients with FPIES compared with control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive findings</strong></td>
</tr>
<tr>
<td>Humoral responses</td>
</tr>
<tr>
<td>Lower levels of milk-specific IgG and IgG4 in patients with CM-FPIES compared with those in patients tolerating CM (active vs resolved)</td>
</tr>
<tr>
<td>Trend toward lower ratios of casein-specific IgG/total IgG in patients with active CM-FPIES and patients with resolved CM-FPIES compared with those in patients with CM-tolerant FPIES</td>
</tr>
<tr>
<td>Lower casein-specific IgM/total IgM ratio in patients with active CM-FPIES compared with that in patients tolerating CM</td>
</tr>
<tr>
<td>Significantly lower milk-specific λ Ig-fLC/κ Ig-fLC ratio in patients with active CM-FPIES compared with that in patients tolerating CM</td>
</tr>
<tr>
<td>T-cell and cytokine responses</td>
</tr>
<tr>
<td>Significantly lower secretion of IL-10 in patients with CM-FPIES compared with that in patients with IgE-CMA</td>
</tr>
<tr>
<td>Significantly higher IL-9-induced secretion in patients with CM-FPIES compared with that in patients with IgE-CMA</td>
</tr>
<tr>
<td>Serum cytokines</td>
</tr>
<tr>
<td>At baseline:</td>
</tr>
<tr>
<td>1. Higher median concentration of IL-10 in patients with a negative OFC result compared with those with a positive OFC result</td>
</tr>
<tr>
<td>2. Higher median IP-10 concentration in patients with a positive OFC result</td>
</tr>
<tr>
<td>Significant increase in IL-10 and IL-8 secretion after a positive OFC result</td>
</tr>
<tr>
<td>Tryptase level before and after an OFC</td>
</tr>
<tr>
<td>Baseline serum tryptase levels significantly higher in patients with FPIES with a positive OFC result compared with levels in those with a negative OFC result</td>
</tr>
<tr>
<td>Serum tryptase levels not significantly different after a positive OFC result in patients with FPIES</td>
</tr>
</tbody>
</table>

MCP-1, Monocyte chemotactic protein 1; MIP, macrophage inflammatory protein.
Humoral and cellular responses to casein in patients with food protein–induced enterocolitis to cow’s milk

Jean Christoph Caubet, MD, a,b Ramon Bencharitiwong, PhD, b Andrew Ross, BA, b Hugh A. Sampson, MD, b M. Cecilia Berin, PhD, b and Anna Nowak-Węgrzyn, MD b Geneva, Switzerland, and New York, NY

JACI 2016
What are the mechanism that cause FPIES

Berin C, JACI 2016.
Can FPIES become IgE mediated allergy?

160 children retrospectively studied
• 39% had IgE sensitization to another food.
• 24% subjects had positive specific IgE levels to the food inducing FPIES.
• Among children with specific IgE to cow’s milk, 41% changed from a milk FPIES to an IgE-mediated phenotype over time.
• none of the subjects with milk specific IgE became tolerant to milk during the study

Caubet et al JACI 2014
Modified from
http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/A/AntigenPresentation.html
MHC class II: Loci HLA-DQ, HLA-DP and HLA-DR, present on antigen presenting cells such as dendritic cells, macrophages, and B cells.

7 studies total = 2 positive 4 negative association

Early studies using candidate gene approaches found associations between PA and HLA-DR and -DQ alleles (HLA-DRB1*08 and DQB1*06:03P) when comparing subjects with peanut allergy with nonallergic unrelated control groups. No significant associations were found between siblings with and without peanut allergy. However, a recent large genomewide association study of patients with peanut allergy and their family members found 2 Paassociated single-nucleotide polymorphisms (rs9275596 and rs7192) mapping to regions involving the HLADR and HLA-DQ genes. Associations with differential DNA methylation partly mediated the associations between PA and single-nucleotide polymorphisms.

Hemler et al. / Ann Allergy Asthma Immunol 115 (2015) 471e476
HLA Major histocompatibility complex (MHC) role in Peanut allergy

Conclusions

• Non-IgE mediated allergies continue to provide unique challenges
• Lack of clear in vitro specific diagnostic test
• Difficulties in the diagnosis of Food allergy that rely heavily on Oral food challenges and EGD make these emerging diseases
• Ongoing studies maybe able to answer some of the questions
Thanks

- ILSI Health & Environmental Sciences Institute (HESI)
- Protein Allergenicity Technical Committee (PATC) for invitation
- All the families and patients that participate to the study
- APFED/AAAAAI
- FARE
- Dr Spergel
Wheat is a cereal grain composed of 4 fractions of proteins (ie, albumins, globulins, and “gluten” [gliadins and glutenins]), any of which might elicit an IgE-mediated allergic response. 16% of IgE mediated allergic patients to wheat react to Rye, up to 16-55% to barely, they usually tolerate oat.

In animal model EoE is Non-IgE mediated food allergy

Noti et al, Nat. Medicine 2013
IgG4 specific for some foods maybe important, but their clinical value is to be determined

Clayton et al Gastroenterology 2014
TSLP-thymic stromal lymphopoietin

progenitors
B-1 cells
IL-13
responses
Basophils
IL-13
Mast-cells
Eosinophils
Recruitment
Dendritic cells
IL-4, Th2
Survival
OX40L
chemokines
↓ Il12, IL23p40

TSLP
IL-13
Cytotoxicity
CD8+ T cells
CD4+ T cells
iNKT
Th2
↓ Il12, IL23p40

IL-12, IL23p40
Thymic stromal lymphopoietin–elicited basophil responses promote eosinophilic esophagitis

Mario Noti1,2,27, Elia D Tait Wojno1,2,27, Brian S Kim1–3, Mark C Siracusa1,2, Paul R Giacomin1,2,4, Meera G Nair1,2,5, Alain J Benitez6, Kathryn R Ruymann7, Amanda B Muir6, David A Hill1,2,7, Kudakwashe R Chikwava8, Amin E Moghaddam9, Quentin J Sattentau9, Aneesh Alex10–12, Chao Zhou10–12, Jennifer H Yearley13, Paul Menard-Katcher14, Masato Kubo15,16, Kazushige Obata-Ninomiya17,18, Hajime Karasuyama17,18, Michael R Comeau19, Terri Brown-Whitehorn7, Rene de Waal Malefyt20, Patrick M Sleiman21–23, Hakon Hakonarson21–23, Antonella Cianferoni7, Gary W Falk14,24,25, Mei-Lun Wang6,24,25, Jonathan M Spergel12,27,24,25 & David Artis1,2,24–26
Eotaxin 3 Expression in EoE

- Examined Gene expression from esophageal biopsies
- Found dysregulation compared to control population
- Eotaxin-3 upregulated 50X in EoE

Candidate gene approach revealed a SNP +2496 T G in Eotaxin 3 Gene predisposed to EoE development in

Which are the cells that produce Th2 cytokines in EoE?

(Strauaman AJ Allergy Clin Immunol 2001;108:954-61.)
Which are the cells that produce IL-13 in EoE?

Desmoglein-1 regulates and esophageal epithelial barrier function in eosinophilic esophagitis
Th2 Inflammation and Epithelial damage

CALPAIN 14

Kottyan 2014 Nature Genetics
SMILEE (first trial for milk patch in treatment of milk induced EoE) Study Design

**Milk Exposure**

Upper Endoscopy/Biopsy

- >15
- <5

**EPIT Treatment**

- Randomization
- Start Treatment

**Milk-Free Diet**

- V1
- V2
- V3
- V4
- V5
- V6
- V7
- V8
- V9
- V10
- V11

**Milk Exposure**

- Up to 11
- 2 weeks after V10

**Primary Endpoint**

End Treatment

* Milk Exposure period is from 1 week to 2 months
1) Other than the OFC, there are no laboratory or other diagnostic procedures specific for diagnosing FPIES.

2) variety of other laboratory tests may be used to support the diagnosis, or even more importantly, to rule out other conditions.

International Consensus Guidelines AAAAI.
A Nowak- Węgrzyn, et al (Submitted)
Humoral and cellular responses to casein in patients with food protein–induced enterocolitis to cow’s milk

A. TNF

B. IL-5

C. IL-6

D. IL-13

E. IFN

F. IL-10

G. IL-9

Jaci, 2016
After Food challenge to cow’s milk, children who developed FPIES acute reactions had Higher IL-10 and IL-8

Jaci, 2016
Mechanism of Food Allergen Sensitization
Failure of Oral Tolerance

Modified Kanao Otsu, 2011
Adverse Reactions to Foods

Toxic reactions
- Food poisoning
- Heavy metal poisoning
- Sgombroid fish poisoning
- Caffeine
- Alcohol
- Histamine toxicity

Nontoxic reactions
- Food intolerance
- Immuno mediated

Food intolerance
- Lactase deficiency
- Galactosemia
- Pancreatic insufficiency
- Gallbladder / liver disease
- Hiatal hernia
- Gustatory rhinitis
- Anorexia nervosa
- Anxiety

Food allergy
Characteristics of Proteins

- Allergens are mainly water soluble glycoproteins 10-70kd
- Are relatively stable to heat, acid and proteases
- Food processing (roasted vs boiled or fried peanuts)
- If animal derived have less than 62% homology with human protein

JENKINS JA JACI, 2007; SICHERER SH ANN REV MED 2009

A limited number of foods are responsible for the majority of the reactions.
Characteristics of Type 1 Food Allergens

Of 9500 contained in PFAM (Protein Family Database of Alignments and Hidden Harvok Model) Most Food allergens belongs to 3 Tropomyosins, EF hands proteins, caseins plus 14 other families with only 1-3 allergens each.

<table>
<thead>
<tr>
<th>Species</th>
<th>Allergenicity*</th>
<th>Sequence accession code</th>
<th>Human TPM1</th>
<th>Shrimp TPM Met e 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human TPM1 (Homo sapiens)</td>
<td>No</td>
<td>P09493</td>
<td>100</td>
<td>52</td>
</tr>
<tr>
<td>Chicken (Gallus gallus)</td>
<td>No</td>
<td>P04268</td>
<td>95</td>
<td>55</td>
</tr>
<tr>
<td>Frog (Rana esculenta)</td>
<td>No</td>
<td>P13105</td>
<td>94</td>
<td>53</td>
</tr>
<tr>
<td>Tuna (Thunnus tonggol)</td>
<td>No</td>
<td>BAD01650</td>
<td>93</td>
<td>53</td>
</tr>
<tr>
<td>Oyster (Crassostrea gigas)</td>
<td>Cra g 1</td>
<td>Q95WY0</td>
<td>44</td>
<td>54</td>
</tr>
<tr>
<td>Abalone (Haliotis diversicolor)</td>
<td>Hal d 1†</td>
<td>Q9GZ71</td>
<td>54</td>
<td>59</td>
</tr>
<tr>
<td>Snail (Helix aspersa)</td>
<td>Hel as 1</td>
<td>O97192</td>
<td>55</td>
<td>60</td>
</tr>
<tr>
<td>Spiny lobster (Panulirus stimpsoni)</td>
<td>Pan s 1</td>
<td>Q61379</td>
<td>53</td>
<td>98</td>
</tr>
<tr>
<td>Crab (Charybdis feriatus)</td>
<td>Cha f 1</td>
<td>Q9NR3</td>
<td>54</td>
<td>81</td>
</tr>
<tr>
<td>Greasybacked shrimp (Metapenaeus ensis)</td>
<td>Met e 1</td>
<td>Q25456</td>
<td>52</td>
<td>100</td>
</tr>
<tr>
<td>Herring worm (Anisakis simplex)</td>
<td>Ani s 3</td>
<td>Q8NAS55</td>
<td>58</td>
<td>69</td>
</tr>
</tbody>
</table>

If similarity with human protein is above 62% there is no allergenicity.
Characteristics of Type 2 Food Allergens

- In PFAM 29 are described allergenic in pollen and 27 in plant food.
- 4 superfamilies of protein account for > 60% of plant food allergens: Prolamine, cupin, Bet v1 homologs, and profilins.

<table>
<thead>
<tr>
<th>Allergen homologous to</th>
<th>Protein classification</th>
<th>Allergen source/allergen</th>
</tr>
</thead>
<tbody>
<tr>
<td>FR-2 type proteins</td>
<td>β-1,3-Glucanases</td>
<td>Fruits, vegetables</td>
</tr>
<tr>
<td>FR-3 type proteins</td>
<td>Basic class I chitinases</td>
<td>Avocado (Pru a 1), chestnut, banana</td>
</tr>
<tr>
<td>FR-4 type proteins</td>
<td>Chitinases similar to potato WH proteins</td>
<td>Tunic, elderberry</td>
</tr>
<tr>
<td>FR-5 type proteins</td>
<td>Thromatin-like proteins</td>
<td>Cherry (Pru av 2), apple (Mal d 2), bell pepper (P23)</td>
</tr>
<tr>
<td>PR-10 type proteins</td>
<td>Bet v 1-homologous proteins</td>
<td>Apple (Mal d 1), cherry (Pru av 1), apricot (Fru ar 1), peach (Pyr c 1), celery (Api g 1), carrot (Dau c 1), parsley (ocPR), potato (1STH)</td>
</tr>
<tr>
<td>PR-14 type proteins</td>
<td>Lipid transfer proteins</td>
<td>Peach (Pru p 3), apple (Mal d 2), soybean (Gly m 1), barley</td>
</tr>
</tbody>
</table>

Protein classification

- Inhibitors of proteases and α-amylases
  - Soybean: Kunitz trypsin inhibitor family, cereals: trypsin/α-amylase inhibitors: barley: Hae v 1/BEA3-1,
  - CpaK, RD1, wheat: CM16-1, egg: Sec e 1, RDAI-1, RDAI-3, rice: RAP

- Peroxidases
  - Wheat, barley

- Prionins
  - Peanut: Ara h 2, soybean: Gly m 5, celery: Api g 4, peach: Pyr c 4, hazelnut, apple, carrot: Lycopersicon, pumpkin, squash

- Seed storage proteins
  - 2S albumins: Yellow mustard: Sin a 1, oriental mustard: Era a 1, oilseed rape: Ball, Brach: Era e 1, English mustard: Era e 1

- Violins
  - Peanut: Ara h 2, English walnut: Jug r 2

- Conglutins
  - Peanut: Ara h 2, Ara h 6, Ara h 7

- Glycinins
  - Peanut: Ara h 2, Ara h 4, soybean

- Beta-conglycinins
  - Soybean

- Thiol-proteases
  - Papaya: papain, fig: ficin, pineapple: bromelain, kiwifruit actinidin/Act e 1, soybean: Gly m 1

- Lectins
  - Peanut: agglutinin
• Heating is only one part of rendering baked milk and egg less allergenic.
• Interactions with proteins, fats, or sugar in a food matrix, such as wheat, are equally important.
• This is why the simple act of boiling CM may not be enough to decrease allergenicity to a degree comparable with a baked product.
• The food matrix may help to reduce exposure of the specific proteins to the immune system.
• For example, the beta-lactoglobulin fractions of whey form disulfide bonds with the other proteins in the food matrix, making them less recognizable by specific IgE.
• OM polymerizes with proteins in the food matrix, such as gluten, to form large insoluble aggregates, making it less recognizable by epitope-specific IgE and potentially less allergenic.
Model of Milk induced inflammation in EoE

Berin CM et al JACI 2008