Non-IgE mediated mechanisms of food allergy

Antonella Cianferoni, MD PhD FAAAAI

DIVISION OF ALLERGY AND IMMUNOLOGY
THE CHILDREN'S HOSPITAL OF PHILADELPHIA
UNIV. OF PENNSYLVANIA,
PHILADELPHIA, PA, USA

Disclosure

- PI in DBV SMILEE trial (protect time to conduct research)
- FARE Co-director of the FARE CHOP Center (protect time to conduct research)
- Sanofi consultants
- AAAAI-BCI secretary -volunteer
- EAACI-EoE IG-Board-volunteer

Adverse Reactions to Foods

Toxic reactions

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

Nontoxic reactions

Food intolerance

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

Immuno mediated

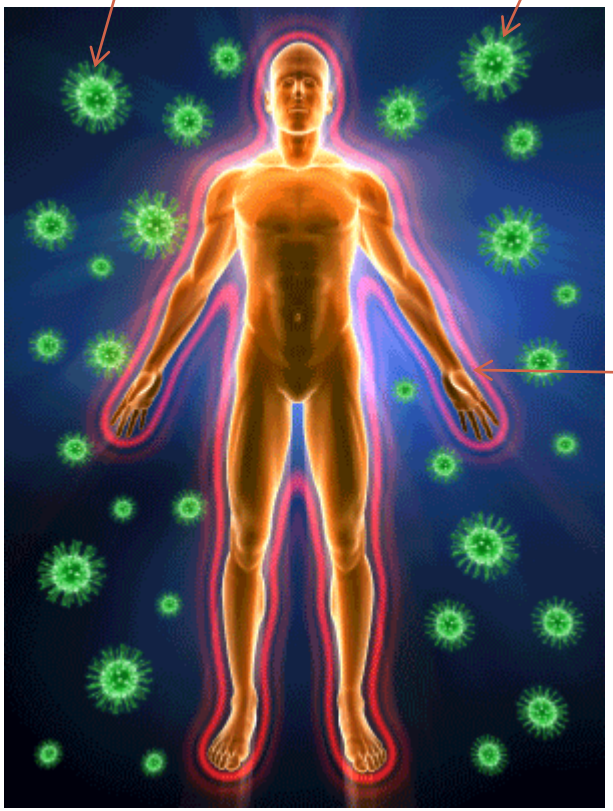
Food allergy

Reproducible immune response to food allergen

Good

viruses

bacteria



Foods

Bad

Immune system

Epithelial cell

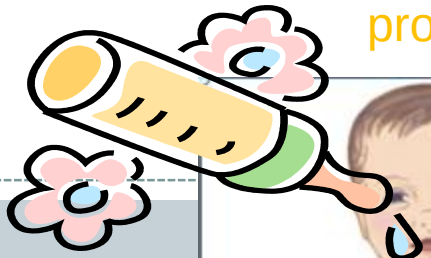
IgE

APC

T cell

Food Antigen Type 1
access to the mucosal immune system

0.75g/kg
protein

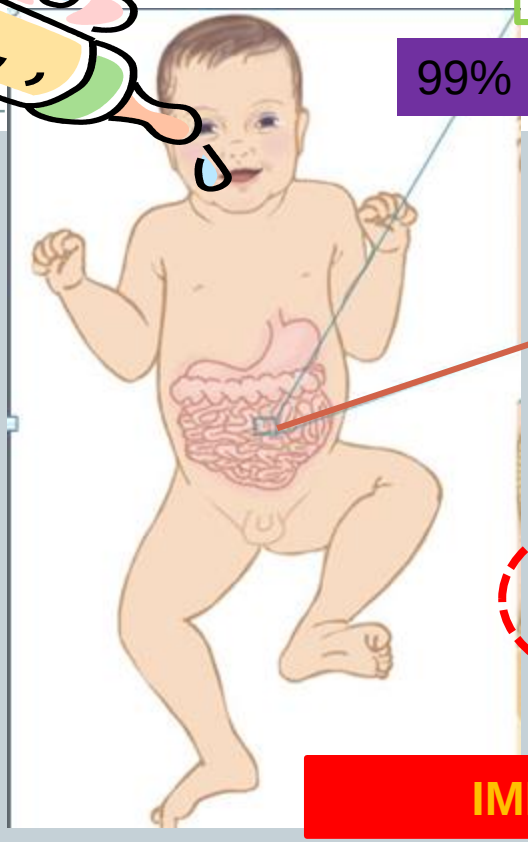


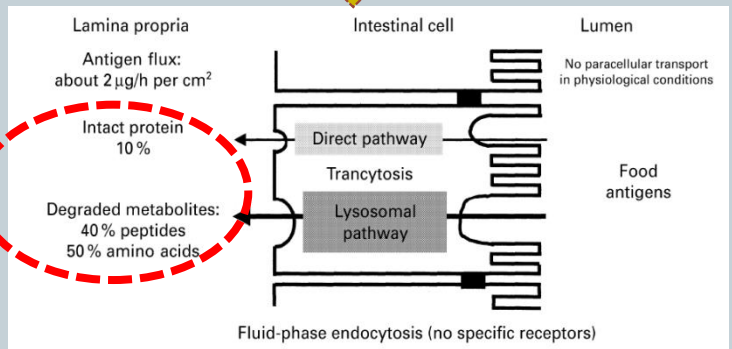
Destruction of immunogenic epitopes by gastric acidity, pancreatic and luminal digestive enzymes

99%

IMMUNOLOGICAL IGNORANCE

1%

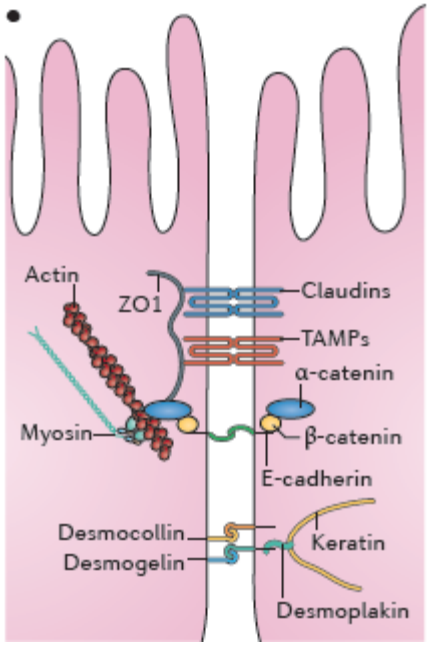




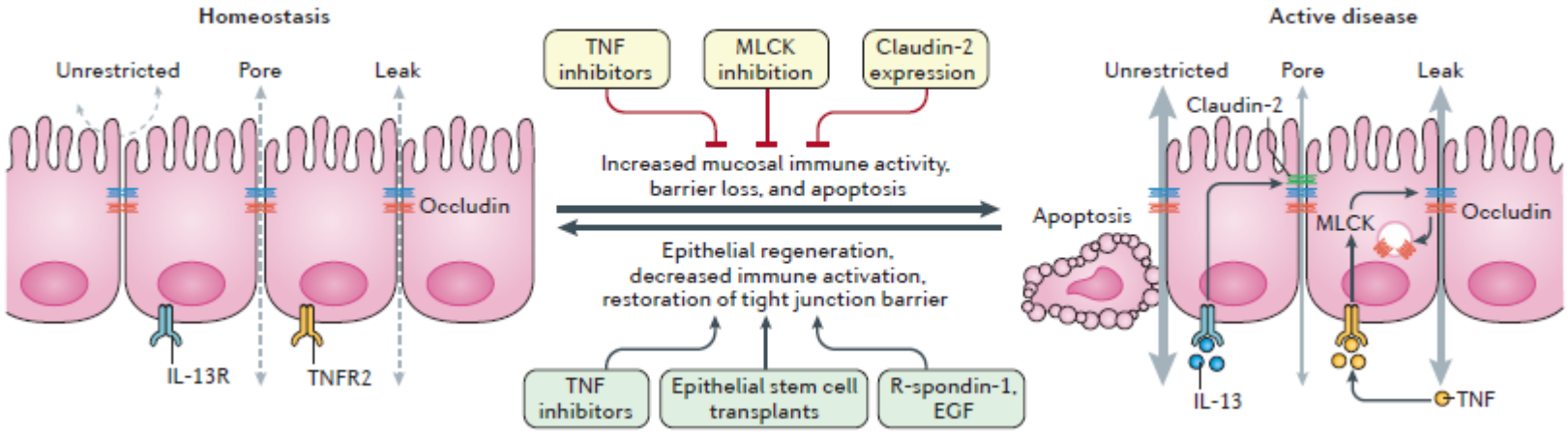
IMMUNOLOGICAL ACTIVE

Chehade M et al JACI 2005,
Heyman M et al Proc Nutr Soc 2001

Epithelial barrier

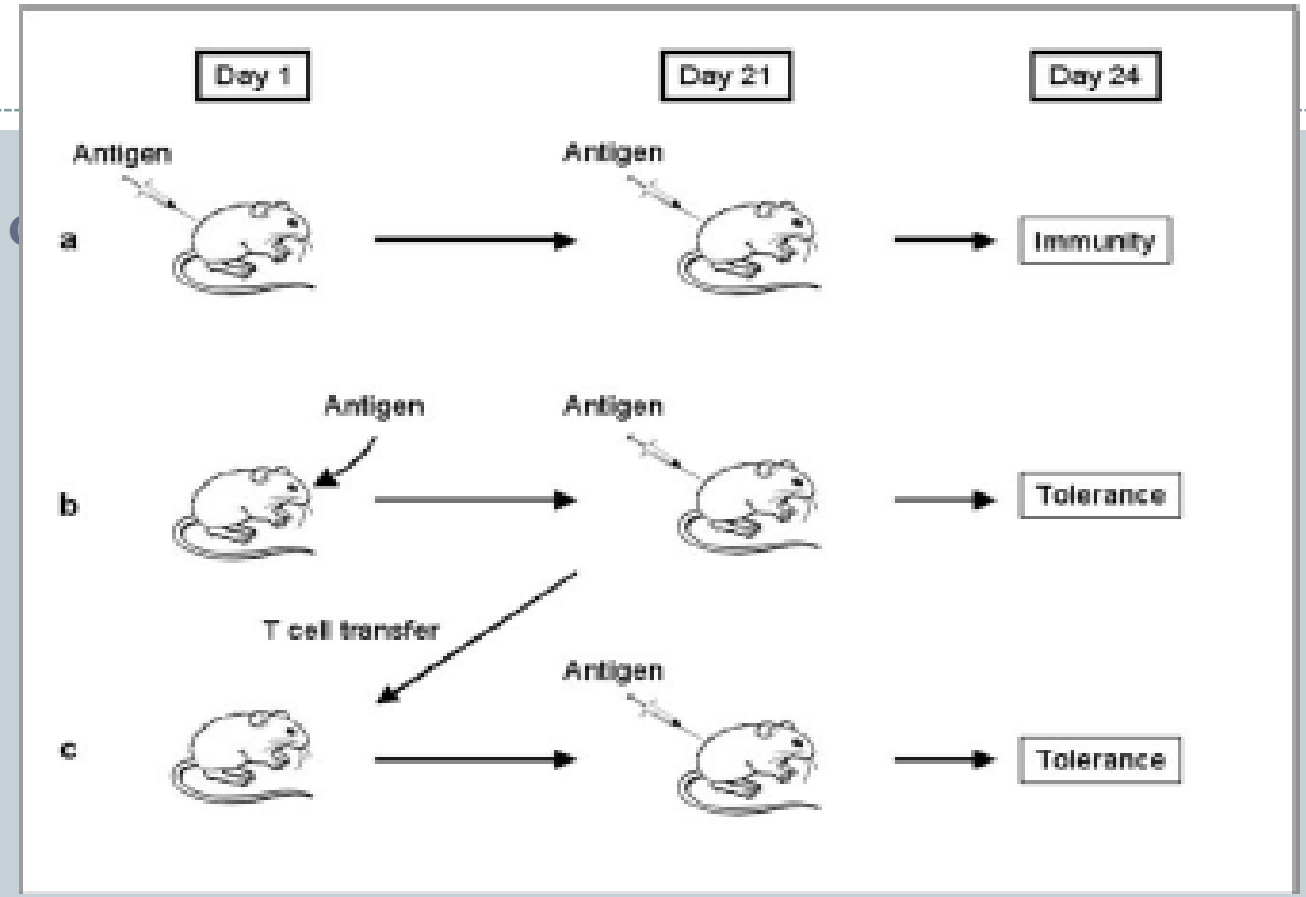


Epithelial cells are held together and communicate through junctions formed by transmembrane proteins
-**tight junction** (claudins and tight junction-associated MARVEL proteins (TAMPs));
-**adherens junction** (E-cadherin)
-**desmosome** (desmogelin and desmocollin)
They are connected to the actin cytoskeleton via cytosolic proteins (ZO1, catenins and desmoplakin).
Tight and adherens junctions interact with the actin cytoskeleton, and desmosomes connect to intermediate filaments.

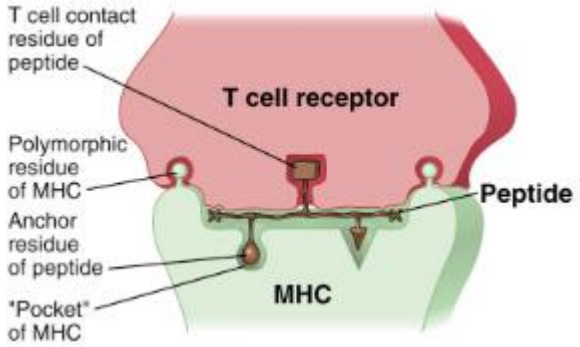


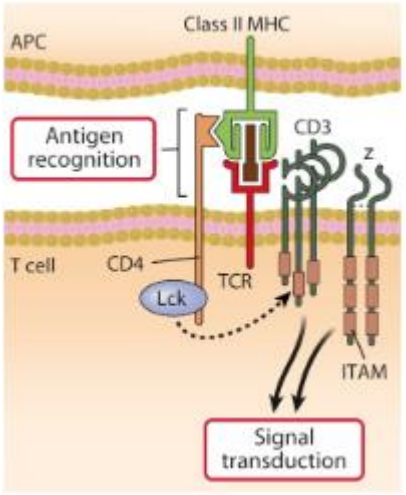
Odenwald MA, 2017

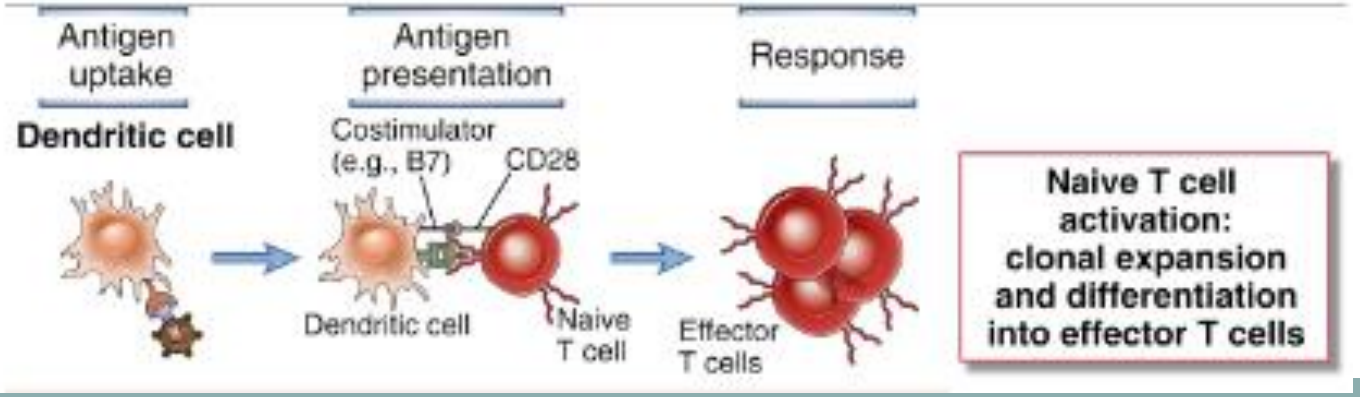
Oral tolerance: role of T cells



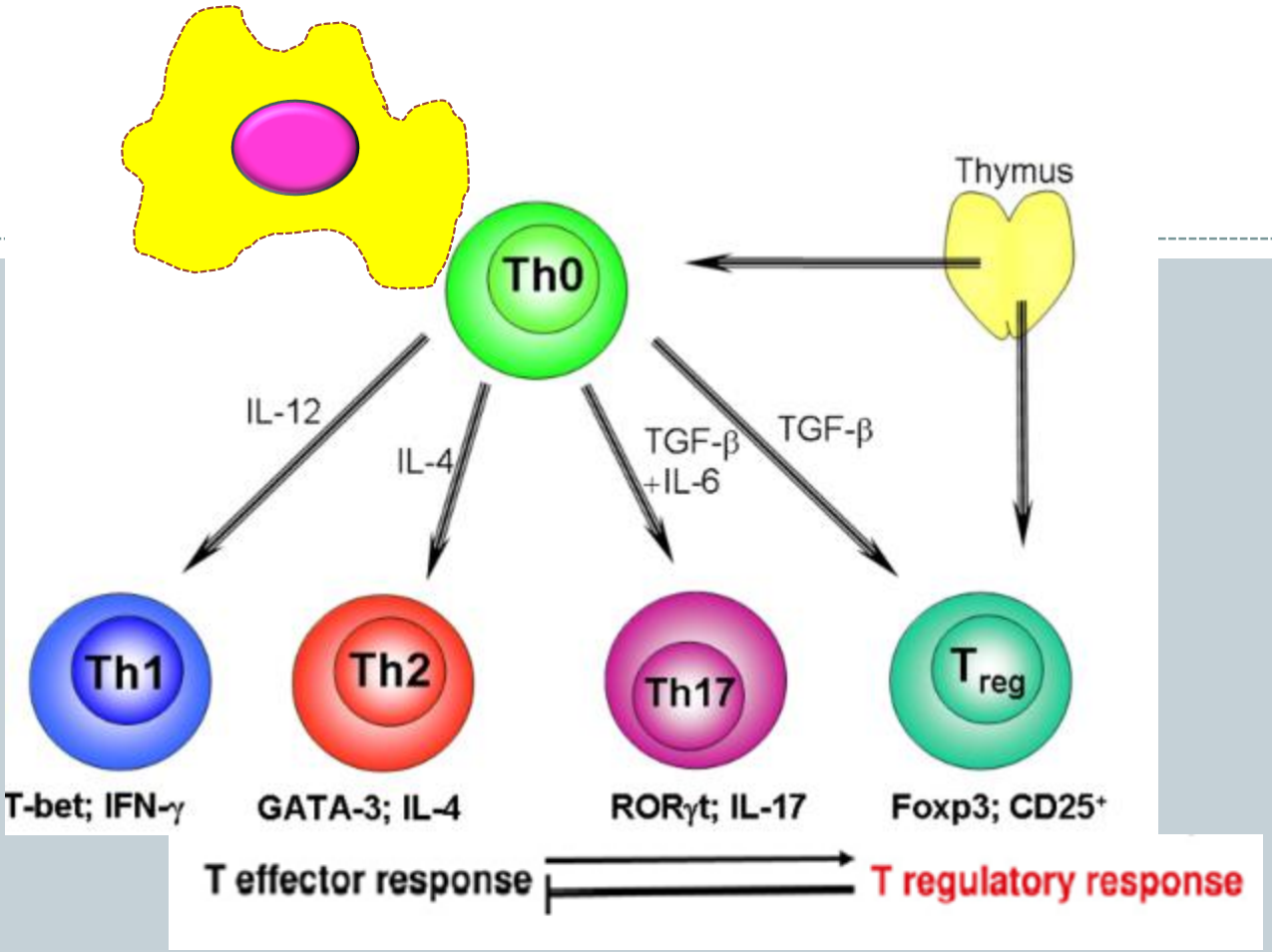
Oral tolerance: role of T cells activation





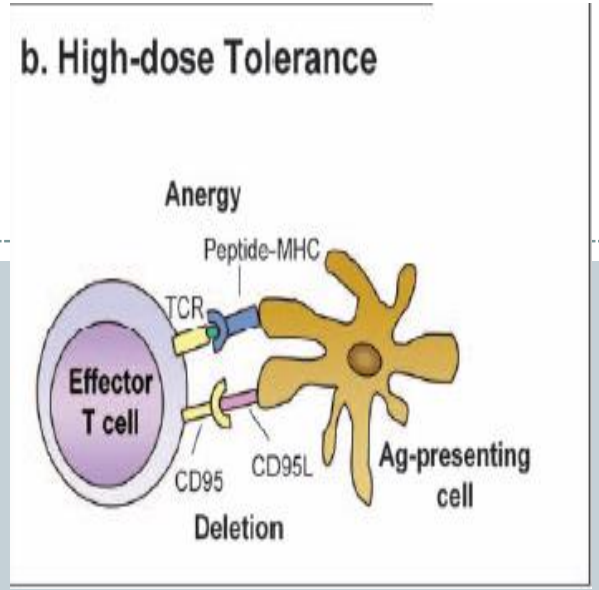


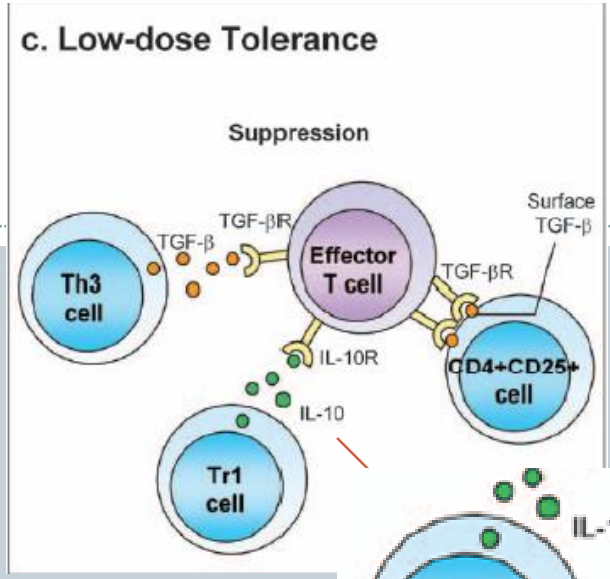
Oral tolerance: Treg in the gut

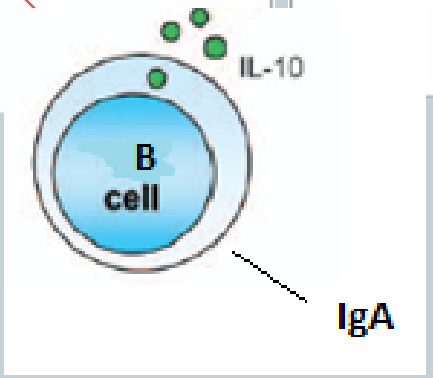


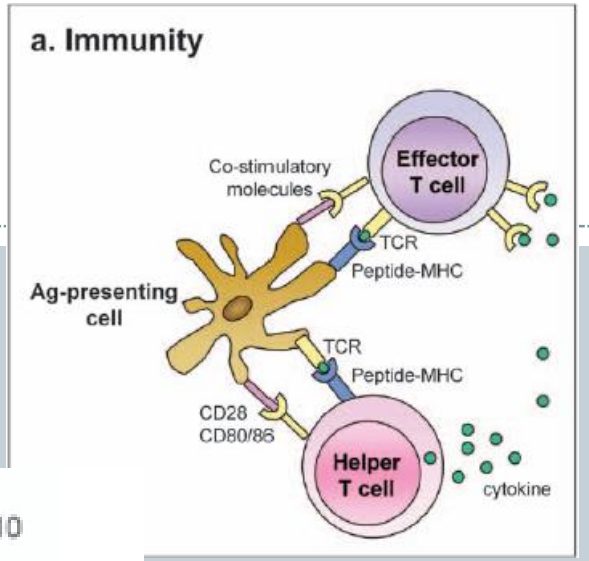
Bacchetta R et al JACI 2007 Chehade M et al JACI 2005

Oral tolerance: role of T cells









$CD4^+$ cells
- T_H3 cells → suppression mainly through secreted TGF-β
- T_R1 cells → suppression mainly through secreted IL-10
- $CD4^+CD25^+$ cells → suppression possibly through surface-bound TGF-β

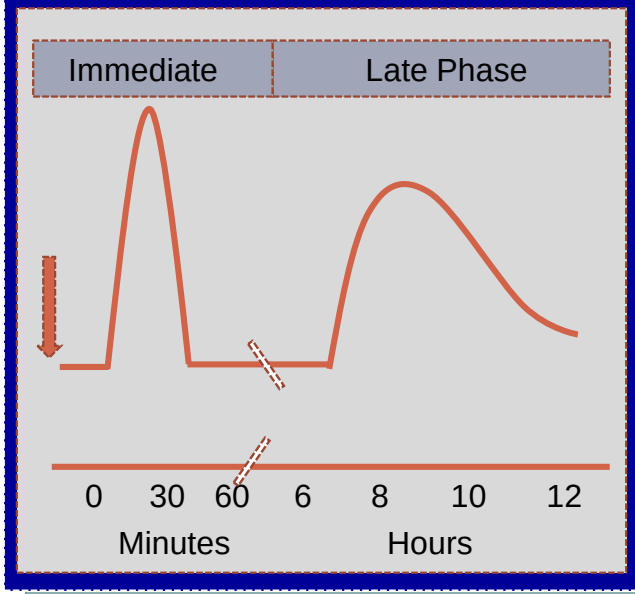
$CD8^+$ cells

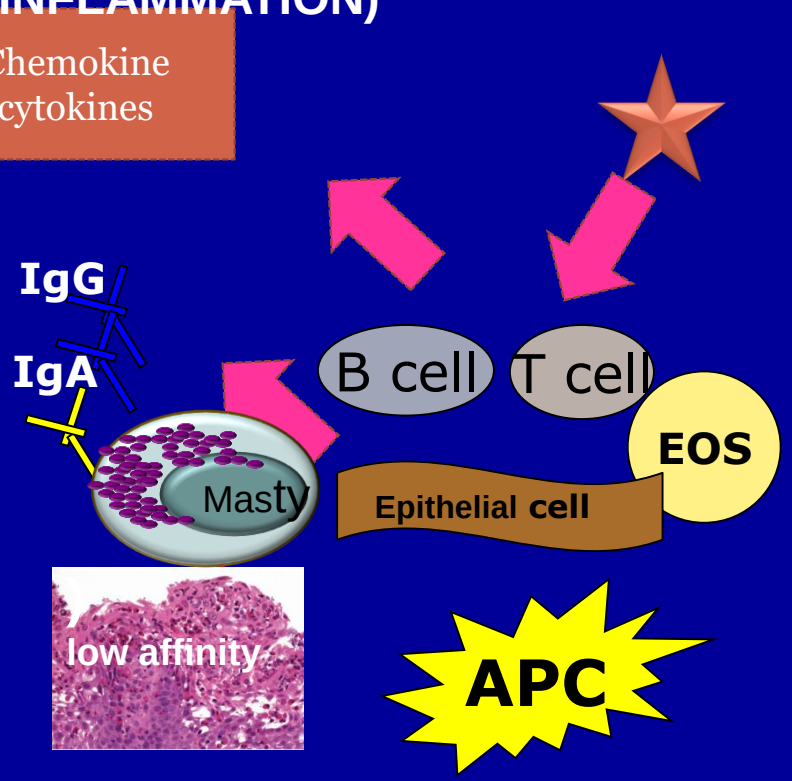
Natural killer T cells

Chehade M et al JACI 2005

Non-IgE mediated food allergy

- Celiac DELAYED REACTION-CHRONIC INFLAMMATION Th1 inflammation
- Eosinophlic Esophagitis (DELAYED REACTION-CHRONIC INFLAMMATION) Th2 inflammation
- FPIES (DELAYED REACTION-ACUTE INFLAMMATION)





Celiac disease (CD) : Definition

- Celiac disease is a chronic, small-intestinal immune mediated enteropathy initiated by exposure to dietary gluten in genetically predisposed individuals and characterized by specific autoantibodies against tissue transglutaminase 2 (anti-tTG2), endomysium, and/or deamidated gliadin peptide
- The genotype *HLA-DQ2* or *HLA-DQ8*, which is required for the development of celiac disease. Although up to 40% of the population carries, only 2%to 3% of *HLA-DQ2* or *HLA-DQ8* carriers subsequently develop celiac disease
- CD can develop at any age and can affect almost any race.
- Prevalence is 1% with regional differences (0-5.6%)
- Some patients such as patients with IgA deficiency, Autoimmune diseases of Thyroid and or Liver, Down Syndrome, Turner Syndrme, IgA nephropathy, Juvenile arthritis, William Syndrome are at higher risk (1.5- 13.5%)

Di Sabatino A, 2009
Leonard MM et al, 2017

Celiac disease (CD) : Manifestation

Intestinal Manifestations (more common in children)

- Children < 3 years : diarrhea, loss of appetite, abdominal distention, and poor growth.
- Children 3 > years or adults : diarrhea, bloating, constipation, abdominal pain, or weight loss.

Extraintestinal Manifestations (due to inflammation, nutrient deficiencies)

- Poor growth, short stature, or delayed puberty (pre puberty)
- Dental enamel defects are (children < 7 years).
- Iron-deficiency anemia (in 32% of adults and 9%of children)
- An increased risk of miscarriage (woman)
- Skin manifestation: dermatitis herpetiformis, urticaria, psoriasis, and dry skin Neurological manifestation or psychiatric manifestationsor both (up to 22% of patients with CD)
- Peripheral neuropathy is frequent, compared with healthy controls (nutritional deficit or inflammation)

Refractory celiac disease is defined as persistent or recurrent malabsorptive symptoms and villous atrophy despite strict adherence to a gluten-free diet for at least 6 -12 months. Uncommon and severe complications such as ulcerative jejunitis and enteropathyassociated T-cell lymphoma

Di Sabatino A, 2009; Leonard MM et al, 2017

Silent coeliac disease

• Patients who do not complain of any symptoms and do not seek medical advice

• Most of these patients are relatives of patients with known coeliac disease or members of the general population found to be positive at the search for antiendomysial antibodies or hTTG antibodies

Minor coeliac disease

• Patients complaining of trivial, transient, or apparently unrelated symptoms (dyspepsia, abdominal discomfort and bloating, mild or occasional altered bowels habit without malabsorption mimicking irritable bowel syndrome, unexplained anaemia, isolated fatigue, cryptic hypertransaminasaemia, infertility, peripheral and central neurologic disorders, osteoporosis, short stature, dental enamel defects, dermatitis herpetiformis), or of isolated symptoms of autoimmune diseases often reported in association with coeliac disease

• Most of these patients are biopsied after positive search of antiendomysial antibodies or hTTG antibodies

Major coeliac disease

• Patients complaining of frank malabsorption symptoms (diarrhoea which is often nocturnal and with incontinence, steatorrhoea suggested by loose discoloured, greasy, and frothy stools that are diffi cult to fl ush away, weight loss and other features of malnutrition, cramps, tetany, and peripheral oedema due to electrolyte and albumin depletion); symptoms of other autoimmune diseases may be associated

• Most of these patients are biopsied only on the basis of symptoms

Di Sabatino A, 2009

Celiac disease (CD) : Diagnosis

Serology: anti-tissue transglutaminase (tTG-IgA), anti-endomysial (EMA-IgA), anti-Deamidated gliadin peptides (DGP-IgG)

Table 3. Range of Sensitivity and Specificity and Use of Current Serologic Tests for Celiac Disease[a]

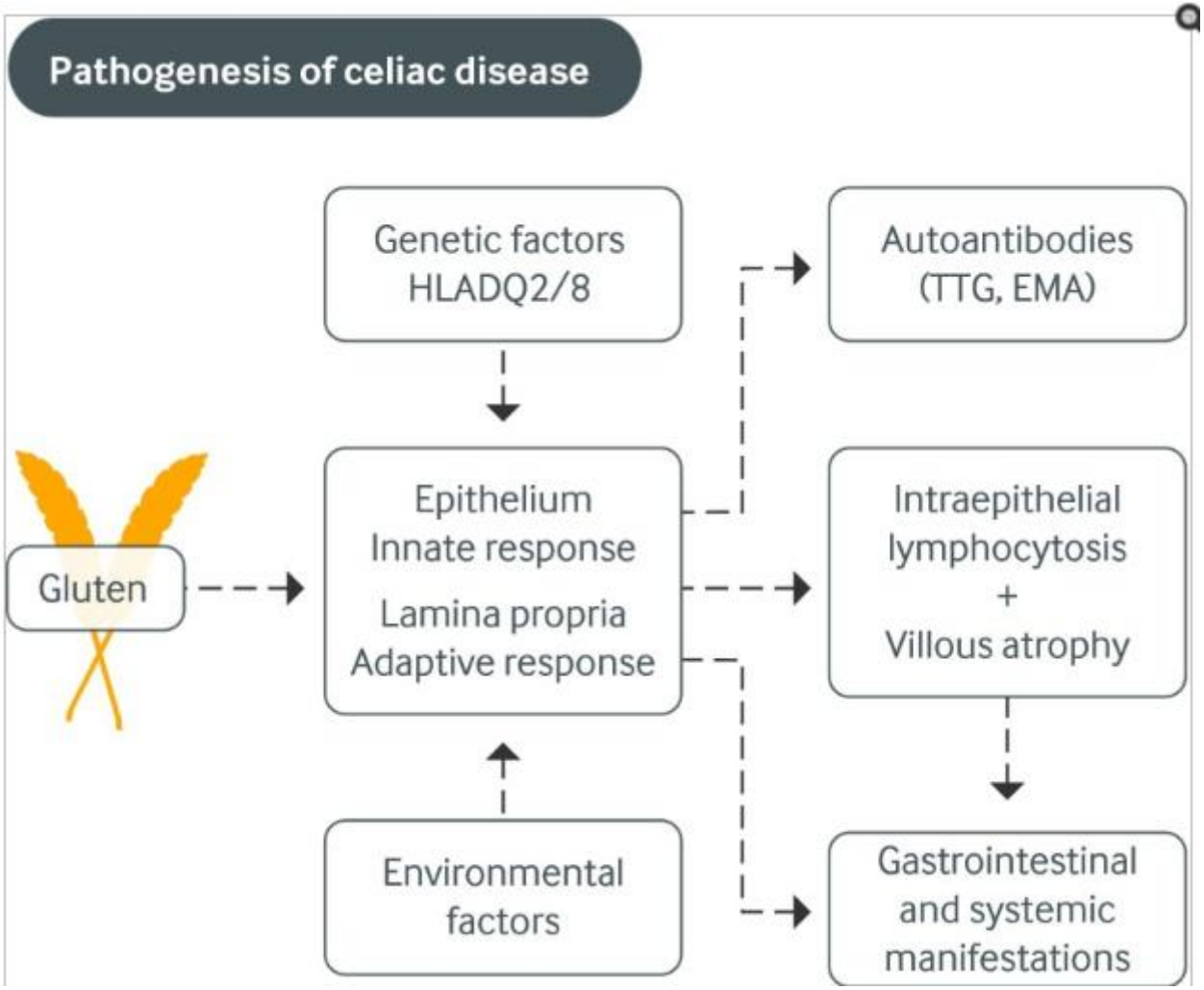
Serologic Study	% Sensitivity	% Specificity	Application in Clinical Practice
IgA tTG	73.9-100	77.8-100	First-line testing to screen for celiac disease[b]
IgG DGP	80.1-96.9	86.0-96.9	First-line testing for celiac disease in patients with IgA deficiency
IgA EMA	82.6-100	94.7-100	Second-line confirmatory test to screen for celiac disease
IgG tTG	12.6-99.3	86.3-100	Not recommended for routine use because of poor sensitivity compared with IgG DGP
IgA DGP	80.7-95.1	86.3-93.1	Not recommended for routine use because of poor sensitivity and specificity compared with IgA tTG and IgA EMA

Biopsy (Duodenum)

- increased number of intraepithelial lymphocytes (>25 per 100 enterocytes)
- elongation of the crypts
- partial to total villous atrophy

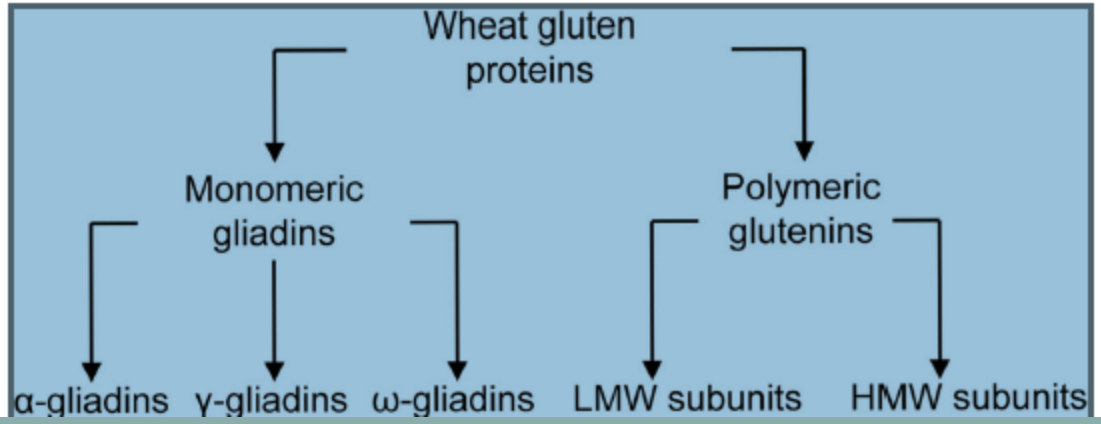
Leonard MM et al, 2017

Celiac Disease: Th1 mediated autoimmune



Celiac Disease: Gluten

Bread wheat (Triticum aestivum) is a globally important food crop
Accounts for 20% of the calories consumed by humans.
It is an important source of protein, vitamins, and minerals.
Storage proteins in wheat are collectively referred to as gluten, give viscosity to dough
Gluten is actually an aggregate formed from two major types of protein:
Gliadin (soluble in 70-90% acqueos alcohol) – prolamin-flow of dough
Glutenin (insoluble in alcohol)-glutelins-elasticity
[Plant proteins are categorized in: albumins, globulins, prolamins, and glutelins].
Glutenin is composed of macropolymers, huge polymers of high- and low-molecular weight subunits crosslinked with disulfide bonds.
These macropolymers intermingle randomly with individual particles of gliadins to form the aggregate, which is held together with non-covalent interactions

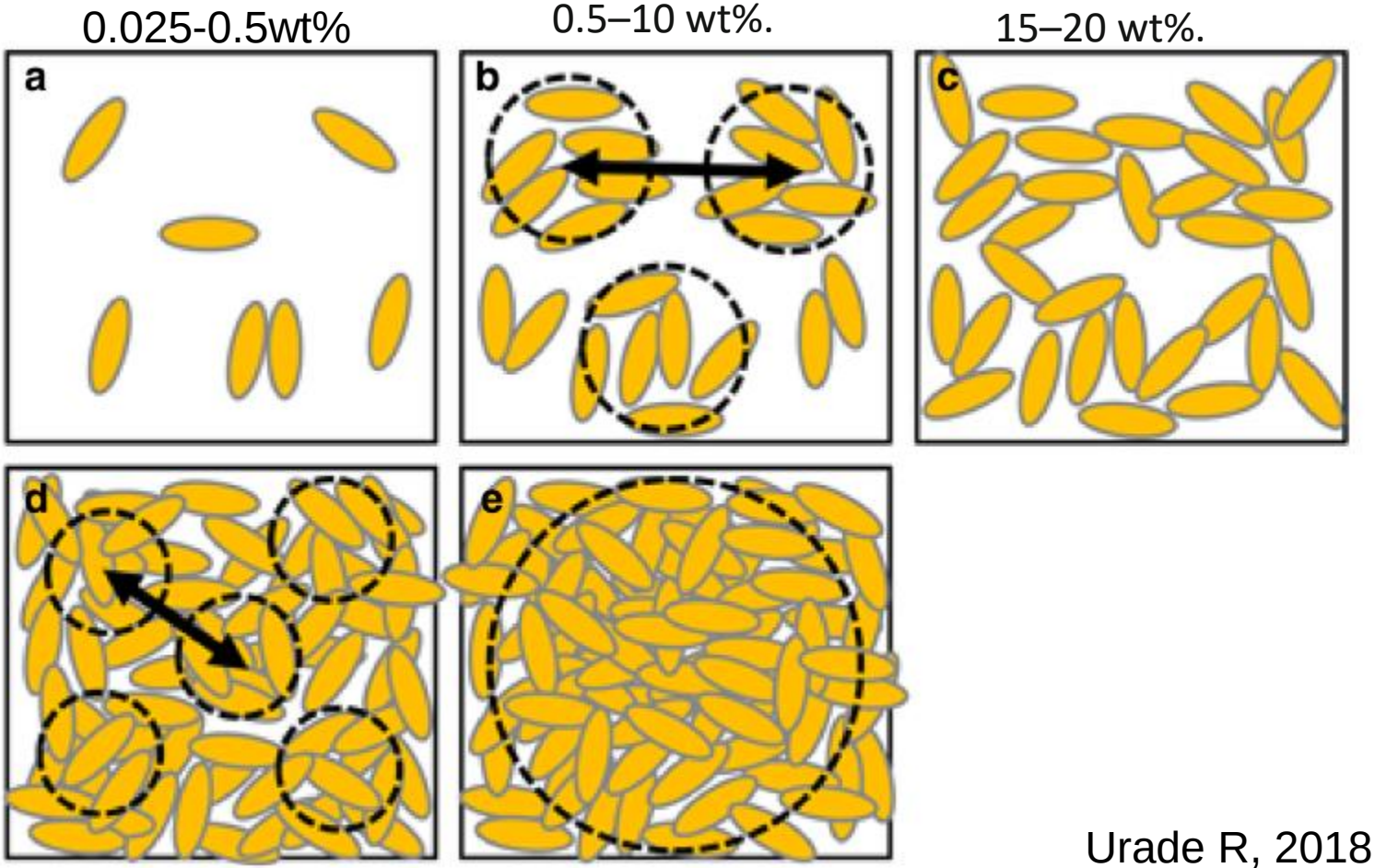


Urade R, 2018

Celiac Disease: Gluten genes

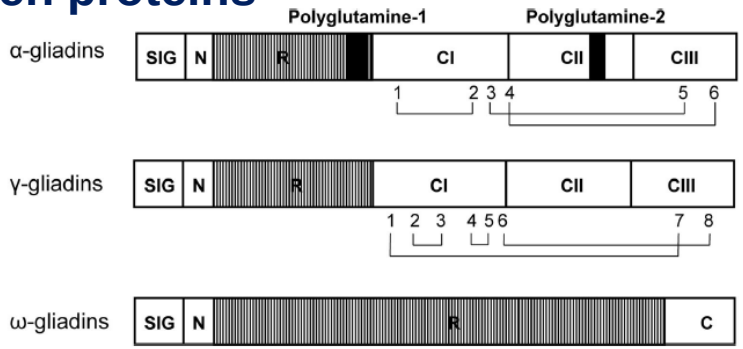
Gluten proteins show extensive polymorphism.
The number of genes encoding gluten proteins increased by duplication and translocation events.
The amino acid sequences of these additional genes have altered due to substitution, deletion, and insertion events during their evolution, apparently in the absence of strong selection pressure.
These changes have resulted in complex mixtures of homologous proteins that vary widely in molecular mass and charge.
This variation has made the isolation and study of these proteins difficult.
Wheat cells have genomes A, B, and D.
γ- and ω-gliadins =Gli-A1, Gli-Bl,and Gli-Dl, located on the short arms of the group 1 chromosomes. γ-gliadins 15–40 copies of genes and ω-gliadins 15–18 copies
The α-and β-gliadins= Gli-A2, Gli-B2, and Gli-D2 on the short arms of the group 2 chromosomes. 25 to 150 copies of the genes (similar so now only α-gliadin)

Gliadins are soluble in pure water when they are at concentrations of less than 10% by weight (wt%). At these concentrations, they yield a transparent solution. When they are in solutions of greater than approximately 15 wt%, gliadins form
gel-like hydrated solids. These will not flow even if the container is inverted at 40 wt% or more



Urade R, 2018

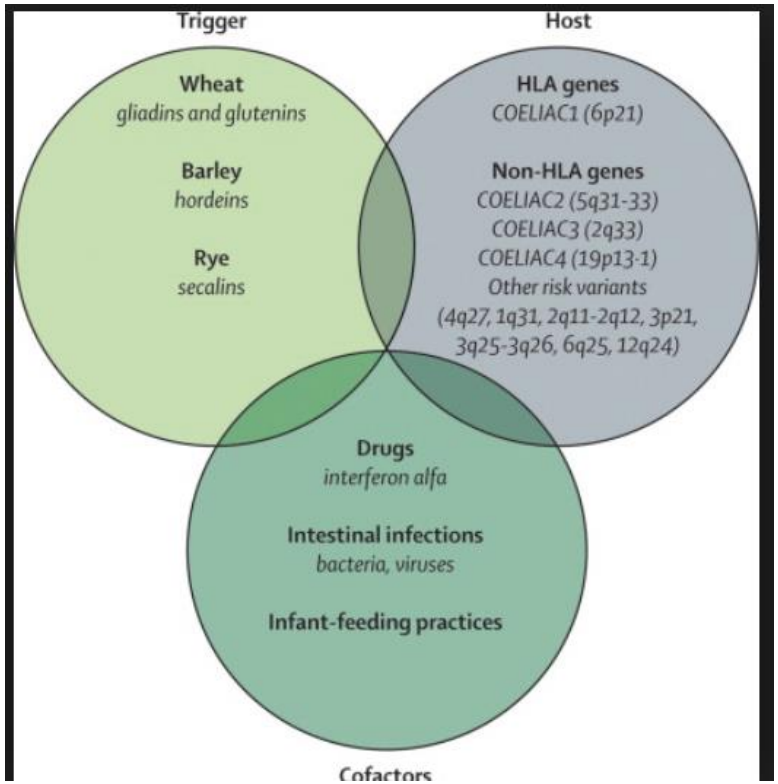
Celiac Disease: Gluten proteins



	α-gliadin (30-34KD)	γ-gliadin (26-36kD)	ω-gliadin
SIG(signal peptide)	20 AA	19 AA	19 AA
N(N-terminal region)	5 residues	12 residues	11 residues
R (repetitive domain)	110-130 residues P(F/Y)PQ3–5.	80-160 residues PFPQQ0–1(PQQ)1–2	238 residues PFPQ1–2PQ1–2
C1 (cysteine rich)	4 cysteine	6 cysteine	No cysteines
CII (glutamine rich)	Glutamine residues	Glutamine res	
CIII	35-39 residues+2 cysteines	41-43 residues+2 cysteines	
Black boxes	2 polyglutamine peptides		

Urade R, 2018

Celiac Disease: Why you develop it?

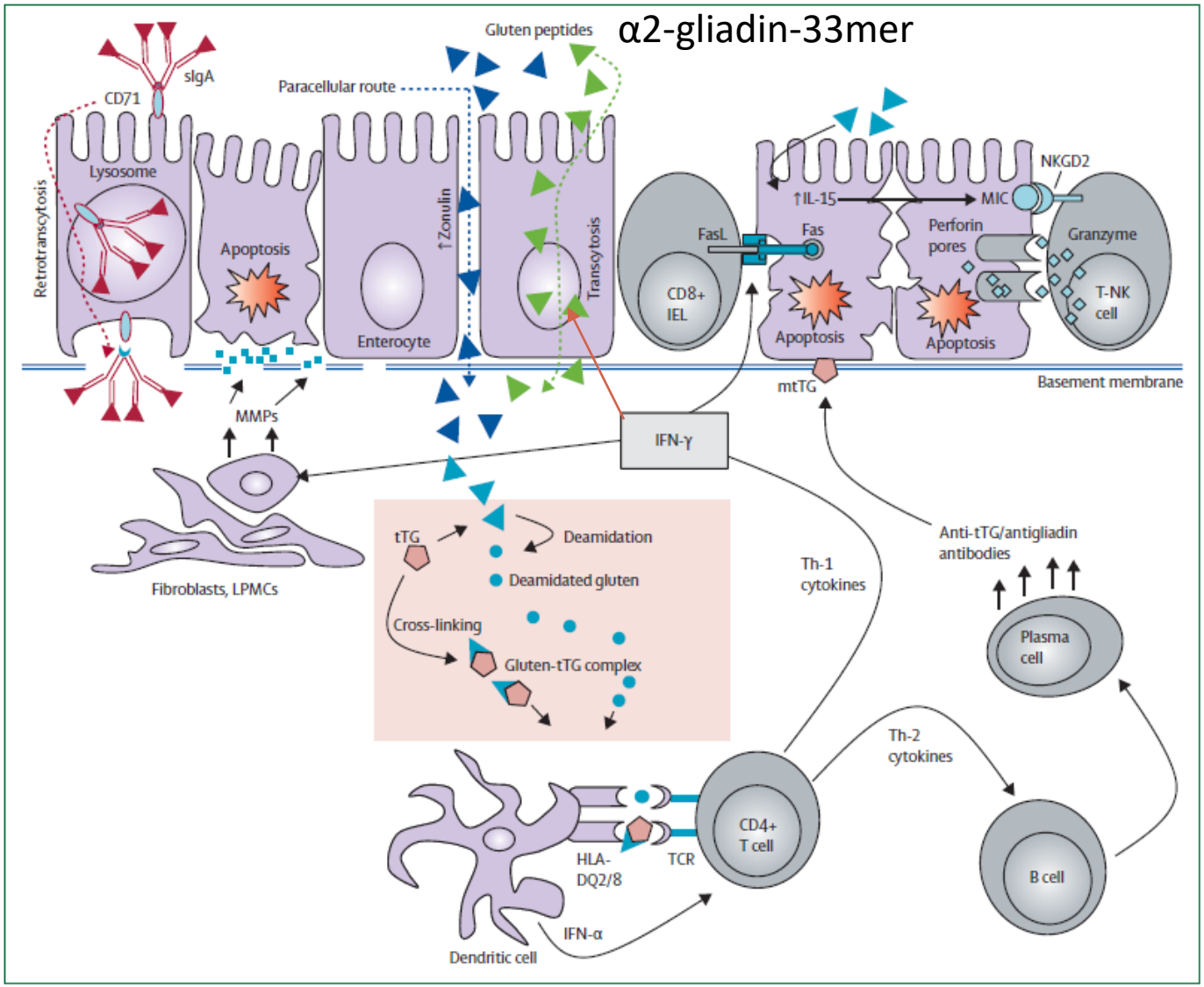


Genetic factors

- basis of familial aggregation
- a concordance rate of about 85% between monozygotic twins
- COELIAC1 6p21-HLA-DQ (strong but mono-zygotes twins stronger than HLA identical)
  - DQ2 (alleles DQA1*05/DQB1*02)
  - DQ8 (alleles DQA1*03/DQB1*0302)
  - DQ2-/DQ8- (DQA1*05 or DQB1*02)
- COELIAC2 (5q31–33) Cytokines
- COELIAC3 (2q33) CTLA4
- COELIAC4 (19p13.1) myosin IXB (alters epithelial actin remodeling)
- GWAS- IL- 2 and IL- 21 (4q27)

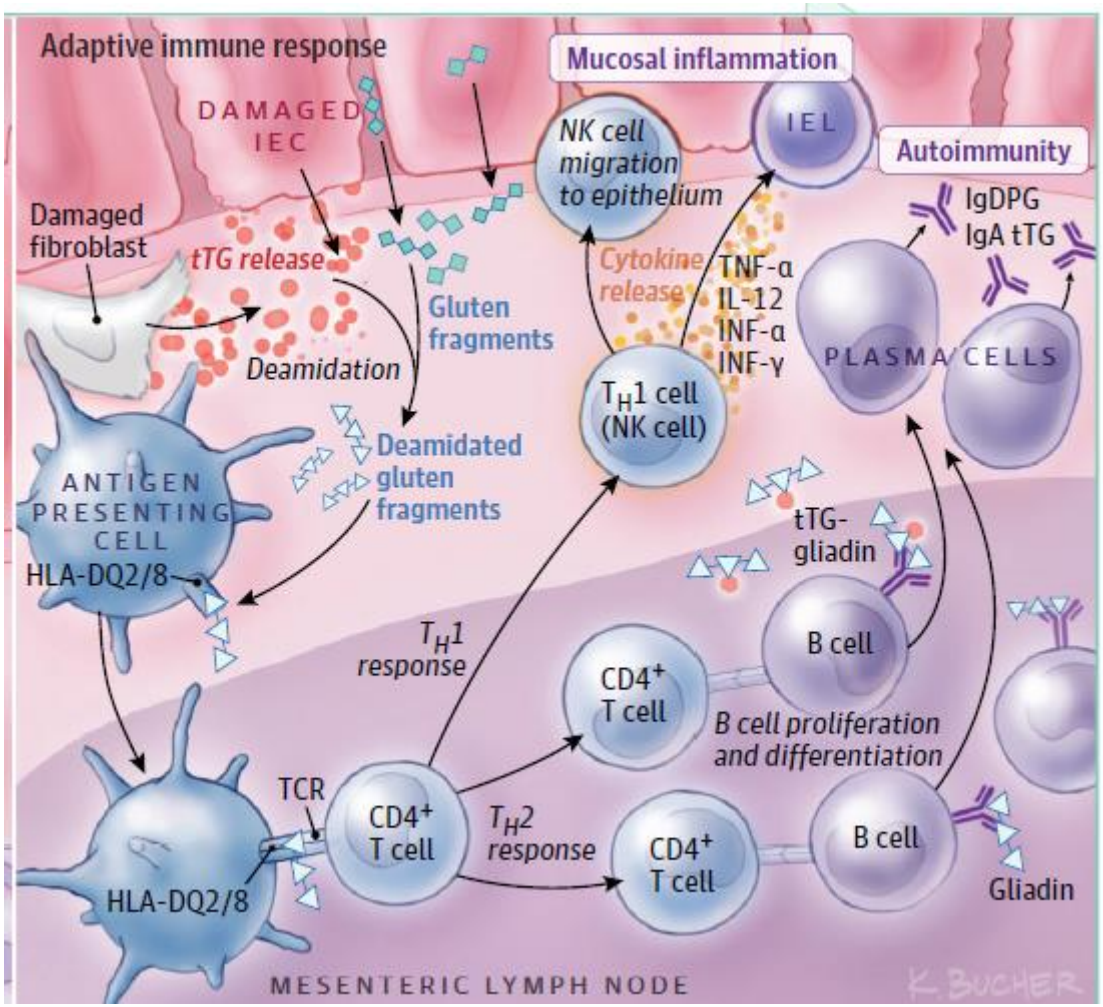
Di Sabatino A, 2009

Celiac Disease: Pathogenesis



Di Sabatino A, 2009

Celiac Disease: Pathogenesis



Leonard MM et al, 2017

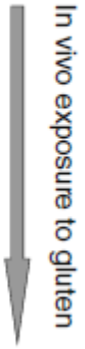
Celiac Disease: immunogenic Peptides

33-mer LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF
(residues 57 to 89) it harbors six partly overlapping DQ2-restricted epitopes

DQ2.5-restricted epitopes	
DQ2.5-glia-α1a	P F P Q P E L P Y
DQ2.5-glia-α1b	P Y P Q P E L P Y
DQ2.5-glia-α2	P Q P E L P Y P Q
DQ2.5-glia-α3	F R P E Q P Y P Q
DQ2.5-glia-γ1	P Q Q S F P E Q Q
DQ2.5-glia-γ2	I Q P E Q P A Q L
DQ2.5-glia-γ3	Q Q P E Q P Y P Q
DQ2.5-glia-γ4a	S Q P E Q E F P Q
DQ2.5-glia-γ4b	P Q P E Q E F P Q
DQ2.5-glia-γ4c	Q Q P E Q P F P Q
DQ2.5-glia-γ4d	P Q P E Q P F C Q
DQ2.5-glia-γ5	Q Q P F P E Q P Q
DQ2.5-glia-ω1	I P F P Q P E Q P F
DQ2.5-glia-ω2	P Q P E Q P F P W
DQ2.5-glut-L1	P F S E Q E Q P V
DQ2.5-glut-L2	F S Q Q Q E S P F
DQ2.5-hor-1	P F P Q P E Q P F
DQ2.5-hor-2	P Q P E Q P F P Q
DQ2.5-hor-3	P I P E Q P Q P Y
DQ2.5-sec-1	P F P Q P E Q P F
DQ2.5-sec-2	P Q P E Q P F P Q
DQ2.5-ave-1	P Y P E Q E E P F
DQ2.5-ave-1b	P Y P E Q E Q P F
DQ8-restricted epitopes	
DQ8-glia-α1	E G S F Q P S Q E
DQ8-glia-γ1a	E Q P Q Q P F P Q
DQ8-glia-γ1b	E Q P Q Q P Y P E
DQ8-glut-H1	Q G Y Y P T S P Q

Risk phenotypes

- High risk: HLA-DQ2.5
  - Large peptide repertoire
  - Largely resistant to degradation
- Low risk: HLA-DQ8
  - Small peptide repertoire
  - Less resistant to degradation
- Very low risk: HLA-DQ2.2
  - Very small peptide repertoire
  - Low resistance to degradation



Shan L, 2002, Koning F, 2012

Non Celiac disease Gluten Sensitivity (NCGS)

The clinical symptoms of nonceliac gluten sensitivity begin after the ingestion of gluten-containing grains.

Symptoms improve or disappear with withdrawal of these grains from the diet, and symptoms reappear after gluten challenge, usually within hours or days.

Gastrointestinal presentation of nonceliac gluten sensitivity is characterized by abdominal pain, bloating, bowel irregularity (diarrhea, constipation, or both),

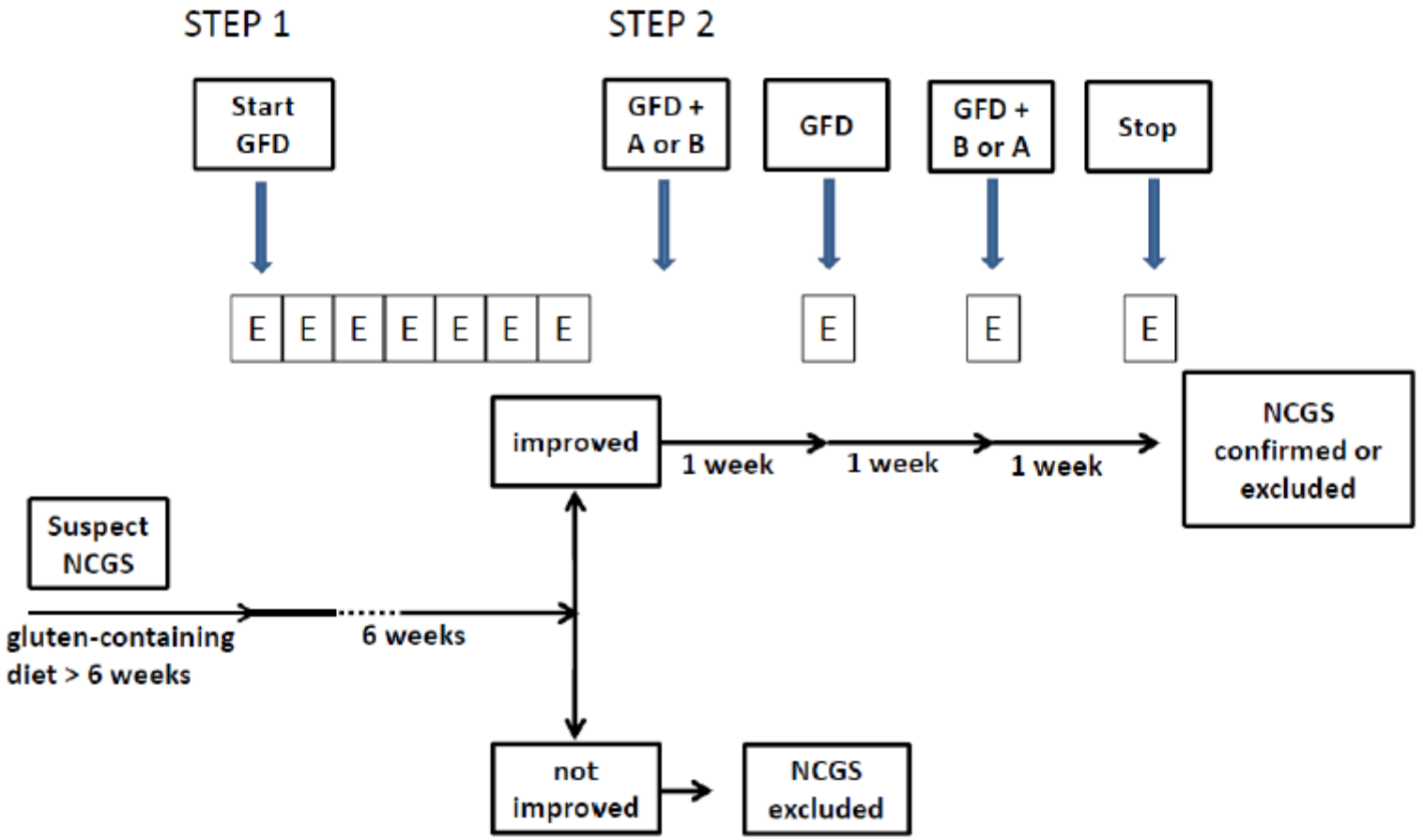
Extra-intestinal manifestations include patient report of a "foggy brain," which is described as slowed thinking, memory disturbance, or reduced level of alertness, along with headache, joint and muscle pain, fatigue, depression, leg or arm numbness, dermatitis (eczema or skin rash),and anemia.

Table 1. The clinical manifestations of Non-Celiac Gluten Sensitivity (NCGS

Frequency	Intestinal	Extra-Intestinal
Very Common	Bloating	Lack of wellbeing
	Abdominal pain	Tiredness
Common	Diarrhea	Headache
	Epigastric pain	Anxiety
	Nausea	Foggy mind
	Aerophagia	Numbness
	GER	Joint/muscle pain
	Aphthous stomatitis	Skin rash/dermatitis
	Alternating bowel habits	
	Constipation	
Undetermined	Hematochezia	Weight loss
	Anal fissures	Anemia
		Loss of balance
		Depression
		Rhinitis/asthma
		Weight increase
		Interstitial cystitis
		Ingrown hairs
		Oligo or polymenorrhea
		Sensory symptoms
		Disturbed sleep pattern
		Hallucinations
		Mood swings
		Autism
		Schizophrenia

Leonard MM et al, 2017

NCGS diagnosis



Effectiveness of gluten free diet in NCGS patients

Author	# of patients	p
Cooper et al 1980	6	P<0.001
Biesiekierski et al 2011	34	P=0.047
Carroccio et al 2012	276	P<0.001
Di Sabatino et al 2015	61	P=0.047

Gluten sensitivity Non-celiac

-34 patients (aged 29-59 years, 4 men) -completed 6 weeks of double blind placebo controlled gluten exposure
-. Overall, 56% had human leukocyte antigen (HLA)-DQ2 and/or HLA-DQ8.
-68% in the gluten group, reported that symptoms were not adequately controlled vs 40% on placebo (P=0.0001).
-On a visual analog scale, patients were significantly worse with gluten within 1 week for overall symptoms (P=0.047), pain (P=0.016), bloating (P=0.031), satisfaction with stool consistency (P=0.024), and tiredness (P=0.001).
-Anti-gliadin antibodies were not induced.
-There were no significant changes in fecal lactoferrin, levels of celiac antibodies, highly sensitive C-reactive protein, or intestinal permeability.
-There were no differences in any end point in individuals with or without DQ2/DQ8.

Biesiekierski JR, 2011

Gluten sensitivity Non-celiac

-36 patients (aged 29-61 years, 6 men)
-completed 2 weeks of fermentable oligo-di-mono-saccharides and polyols (FODMAPs).

then

-1 group did gluten free diet
-1 group low gluten diet
-1 group high gluten diet

All had similar increase of symptoms

Biesiekierski JR, 2011

FODMAPS: **F**ermentable –**O**ligosaccharides – **D**isaccharides –**M**onosaccharides –**A**nd **P**olyols

FODMAPs comprise:
-oligosaccharides, including fructans and galacto-oligosaccharides;
-disaccharides, including lactose;
-monosaccharides, including fructose;
-polyols, including sorbitol, xylitol, and mannitol.

FODMAPS: **F**ermentable –**O**ligosaccharides – **D**isaccharides –**M**onosaccharides –**A**nd **P**olyols

Sources of fructans wheat (although some wheat strains such as spelt contain lower amounts), rye, barley, onion, garlic, Jerusalem and globe artichoke, asparagus, beetroot, chicory, dandelion leaves, leek, radicchio, the white part of spring onion, broccoli, brussels sprouts, cabbage, fennel and prebiotics such as fructooligosaccharides (FOS), oligofructose and inulin]

Sources of galactans Pulses and beans are the main dietary sources (though green beans, tofu and tempeh contain comparatively low amounts

Sources of polyols Polyols are found naturally in some fruit (particularly stone fruits), including apples, apricots, avocados, blackberries, cherries, lychees, nectarines, peaches, pears, plums, prunes, watermelon and some vegetables, including cauliflower, mushrooms and mange-tout peas. They are also used as bulk sweeteners and include isomalt, maltitol, mannitol, sorbitol and xylitol

Fructose and lactose People following a low-FODMAP diet may be able to tolerate moderate amounts of fructose and lactose, particularly if they have lactase persistence

Eliminate foods containing fodmaps

excess fructose

fruit
apple, mango, nashi, pear, tinned fruit in natural juice, watermelon

sweeteners
fructose, high fructose corn syrup

large total fructose dose
concentrated fruit sources, large serves of fruit, dried fruit, fruit juice

honey
corn syrup, fruisana

lactose

milk
milk from cows, goats or sheep, custard, ice cream, yoghurt

cheeses
soft unripened cheeses eg. cottage, cream, mascarpone, ricotta

fructans

vegetables
artichoke, asparagus, beetroot, broccoli, brussels sprouts, cabbage, eggplant, fennel, garlic, leek, okra, onion (all), shallots, spring onion

cereals
wheat and rye, in large amounts eg. bread, crackers, cookies, couscous, pasta

fruit
custard apple, persimmon, watermelon

miscellaneous
chicory, dandelion, inulin, pistachio

galactans

legumes
baked beans, chickpeas, kidney beans, lentils, soy beans

polyols

fruit
apple, apricot, avocado, blackberry, cherry, longon, lychee, nashi, nectarine, peach, pear, plum, prune, watermelon

vegetables
cauliflower, green capsicum (bell pepper), mushroom, sweet corn

sweeteners
sorbitol (420)
mannitol (421)
isomalt (953)
maltitol (965)
xylitol (967)

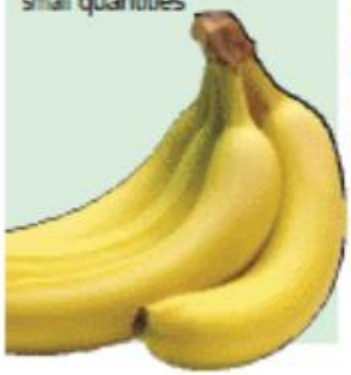
Foods suitable on a low-fodmap diet

fruit

fruit

banana, blueberry, boysenberry, canteloupe, cranberry, durian, grape, grapefruit, honeydew melon, kiwifruit, lemon, lime, mandarin, orange, passionfruit, pawpaw, raspberry, rhubarb, rockmelon, star anise, strawberry, tangelo

Note: if fruit is dried, eat in small quantities



vegetables

vegetables

alfalfa, bamboo shoots, bean shoots, bok choy, carrot, celery, choko, choy sum, endive, ginger, green beans, lettuce, olives, parsnip, potato, pumpkin, red capsicum (bell pepper), silver beet, spinach, squash, swede, sweet potato, taro, tomato, turnip, yam, zucchini

herbs

basil, chili, coriander, ginger, lemongrass, marjoram, mint, oregano, parsley, rosemary, thyme

grain foods

cereals

gluten-free bread or cereal products

bread

100% spelt bread

rice

oats

polenta

other

arrowroot, millet, psyllium, quinoa, sorgum, tapioca

milk products

milk

lactose-free milk*, oat milk*, rice milk*, soy milk*

*check for additives

cheeses

hard cheeses, and brie and camembert

yoghurt

lactose-free varieties

ice-cream substitutes

gelati, sorbet

butter substitutes

olive oil

other

tofu

sweeteners

sugar* (sucrose), glucose, artificial sweeteners not ending in '-ol'

honey substitutes

golden syrup*, maple syrup*, molasses, treacle

*small quantities

Monash University "Low FODMAP Diet".

Vegetables: alfalfa, bean sprouts, green beans, bok choy, capsicum (bell pepper), carrot, chives, fresh herbs, choy sum, cucumber, lettuce, tomato, zucchini
Fruits: banana, orange, grapes, melon
Protein: meats, fish, chicken, tofu, tempeh
Dairy: lactose-free milk, lactose-free yoghurts, hard cheese
Breads and cereals: gluten-free bread and sourdough spelt bread, crisped rice, oats, gluten-free pasta, rice, quinoa
Biscuits (cookies) and snacks: gluten-free biscuits, rice cakes, corn thins
Nuts and seeds: almonds (no more than 10 nuts per serving), pumpkin seeds
Beverage options: water, coffee, tea

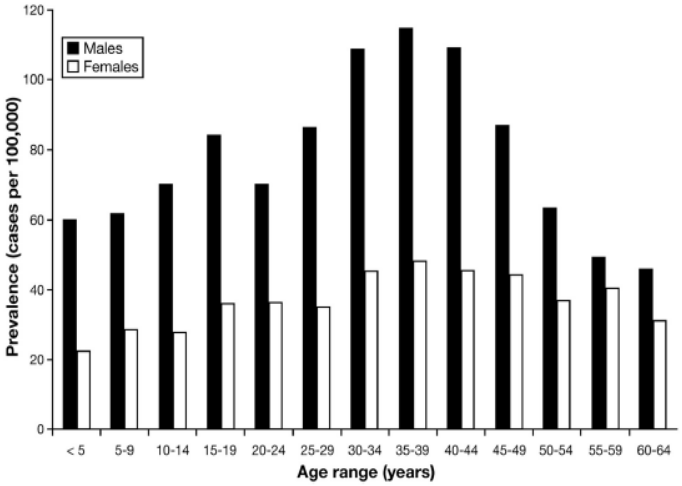
Center for Pediatric Eosinophilic Disorders (CPED)

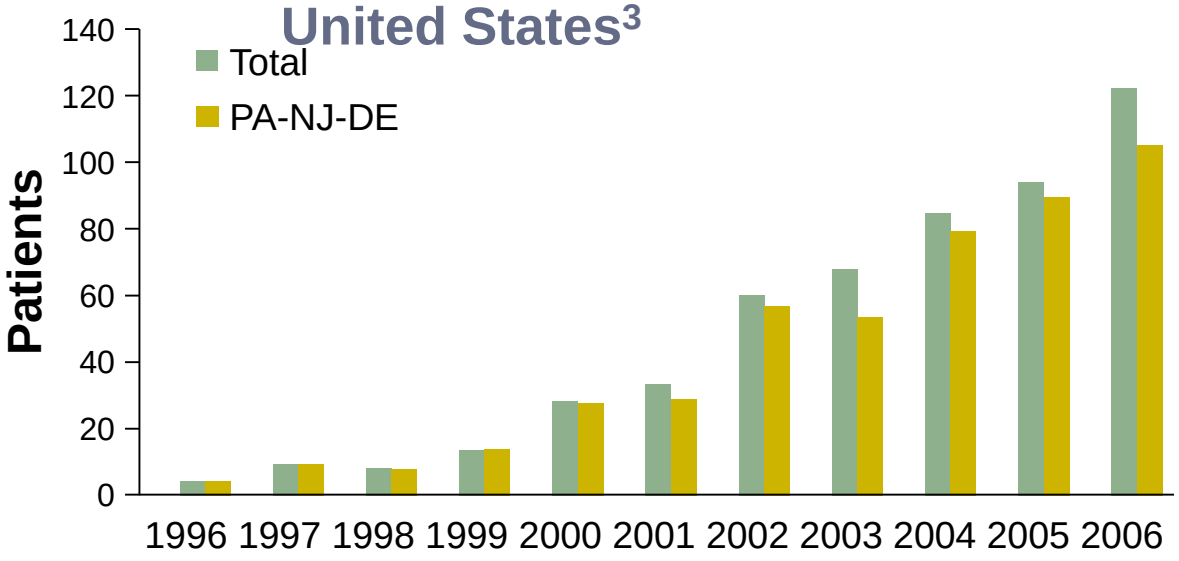
- The largest clinical center in the world with over 1800 patients with EoE

- 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)

EoE: Epidemiology

EoE prevalence 57/100,000





1. Dellon ES, et al. *Aliment Pharmacol Ther.* 2015;41:662-670. **2.** Hruz P. *Dig Dis*. 2014;32:40-47. **3.** Spergel JM, et al. *J Pediatr Gastroenterol Nutr.* 2009;48:30-36.

EoE definition

Clinicopathologic diagnosis

-Presence of clinical symptoms related to esophageal dysfunction

- Dysphagia, vomiting, abdominal pain, heartburn, feeding difficulty, etc.

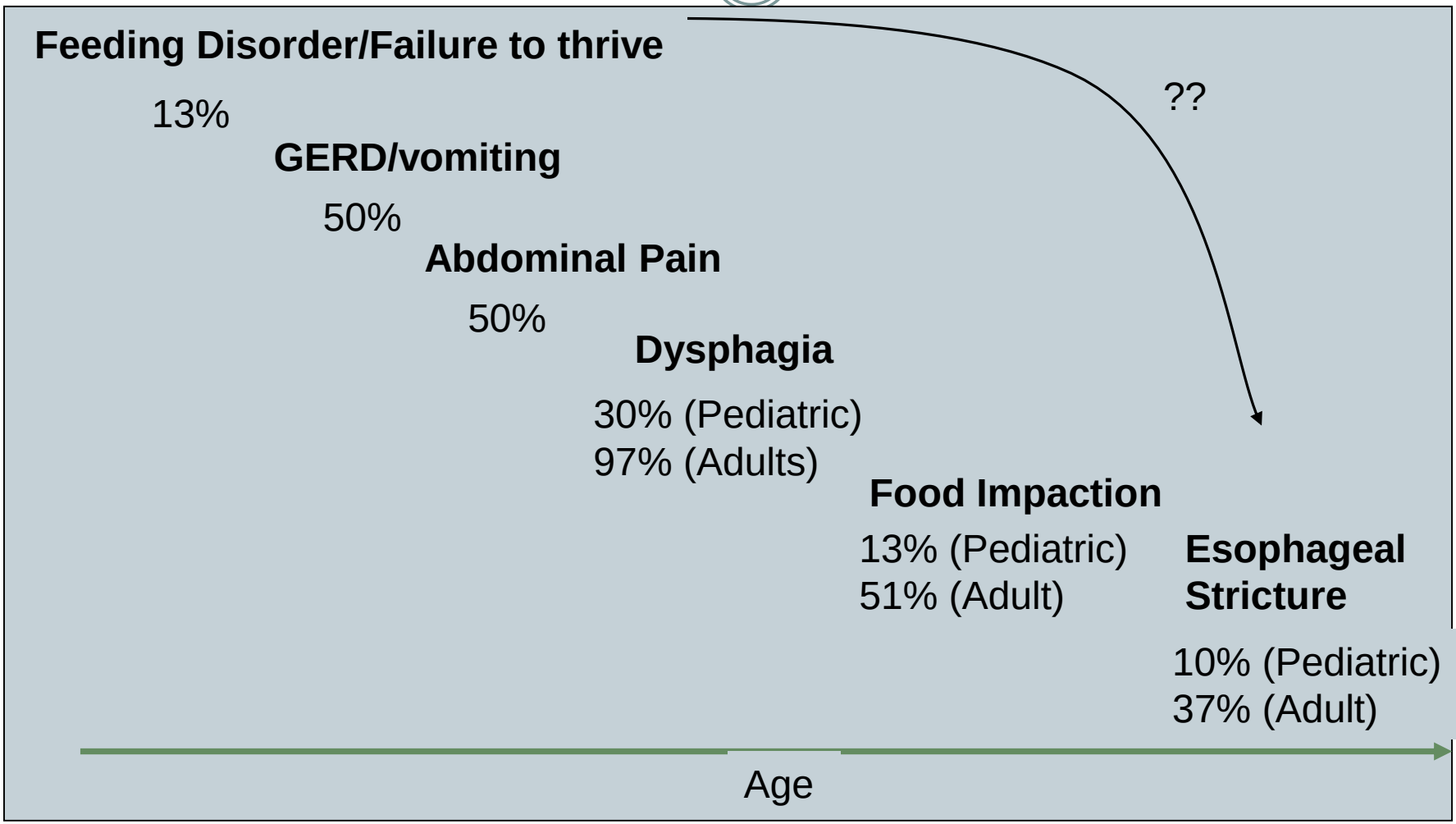
-Isolated esophageal eosinophilia

- **15 or more eosinophils per hpf in at least one esophageal biopsy**
- **Histology of remainder of GI tract normal**

DIAGNOSIS AND FOLLOW UP BASED ON REPETITIVE EGDs

Furuta, et al; Gastroenterology 2007; 133:1342.

Symptom Progression in EoE



Quality of Life EoE is reduced and improved with treatment

1) subject age, EoE symptom burden, atopic comorbidities, and treatment type were associated with base line 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.

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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

Is there a biomarker for EoE?

- Peripheral Biomarkers
  - FeNO, Eotaxin-3, Eosinophilic Proteins, Cytokines, Chemokines, siRNA, stool samples-none identified yet.

- Molecular Signature—Rothenberg Lab
  - Group of genes can distinguish EoE (But still need a biopsy)
    - 1607 significantly dysregulated transcripts (1096 upregulated, 511 downregulated) on RNA seq (Sherrill et al. Genes Immunity 2014)
    - 96 gene panel (EDP) –Wen et al. Gastro 2013

Do Symptoms match Histology?

Table 4. Accuracy of Patient-Reported Clinical Symptoms (Assessed Using EEsAI PRO Score) to Detect Histologic Remission

EEsAI PRO score cutoff	Cumulative remission,[a] n (%)	Cumulative frequency,[b] n (%)	PPV, %	NPV, %	Sensitivity, %	Specificity, %	Accuracy, %
Histologic remission: peak count of <20 eosinophils/mm^2							
15	38/96 (39.6)	96/269 (35.7)	39.6	78.6	50.7	70.1	64.7
20	42/111 (37.8)	111/269 (41.3)	37.8	79.1	56.0	64.4	62.1
25	42/112 (37.5)	112/269 (41.6)	37.5	79.0	56.0	63.9	61.7
30	51/162 (31.5)	162/269 (60.2)	31.5	77.6	68.0	42.8	49.8
35	54/180 (30.0)	180/269 (66.9)	30.0	76.4	72.0	35.1	45.4
Histologic remission: peak count of <60 eosinophils/mm^2							
15	49/96 (51.0)	96/269 (35.7)	51.0	70.5	49.0	72.2	63.6
20	54/111 (48.6)	111/269 (41.3)	48.6	70.9	54.0	66.3	61.7
25	54/112 (48.2)	112/269 (41.6)	48.2	70.7	54.0	65.7	61.3
30	68/162 (42.0)	162/269 (60.2)	42.0	70.1	68.0	44.4	53.2
35	71/180 (39.4)	180/269 (66.9)	39.4	67.4	71.0	35.5	48.7

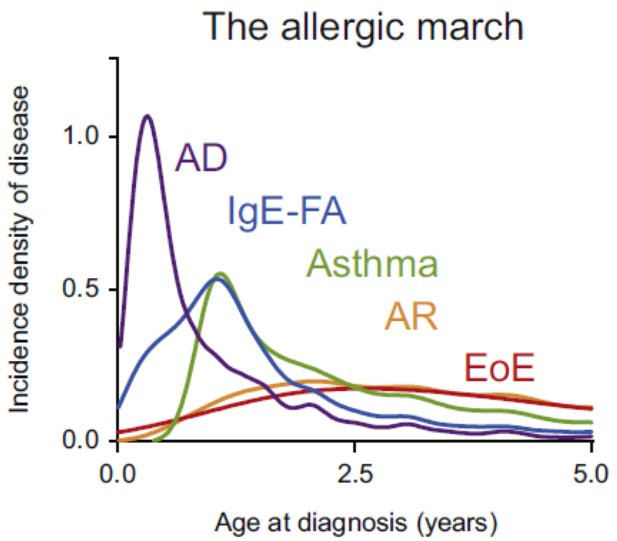
NOTE. Data for 5 different cutoff values of EEsAI PRO score are shown.
NPV, negative predictive value; PPV, positive predictive value.
[a]Number of patients in histologic remission for a given EEsAI PRO score cutoff value.
[b]Number of patients with a given EEsAI PRO score below the cutoff value.

Safroneeva et al. Gastro 2016

Atopy in EoE

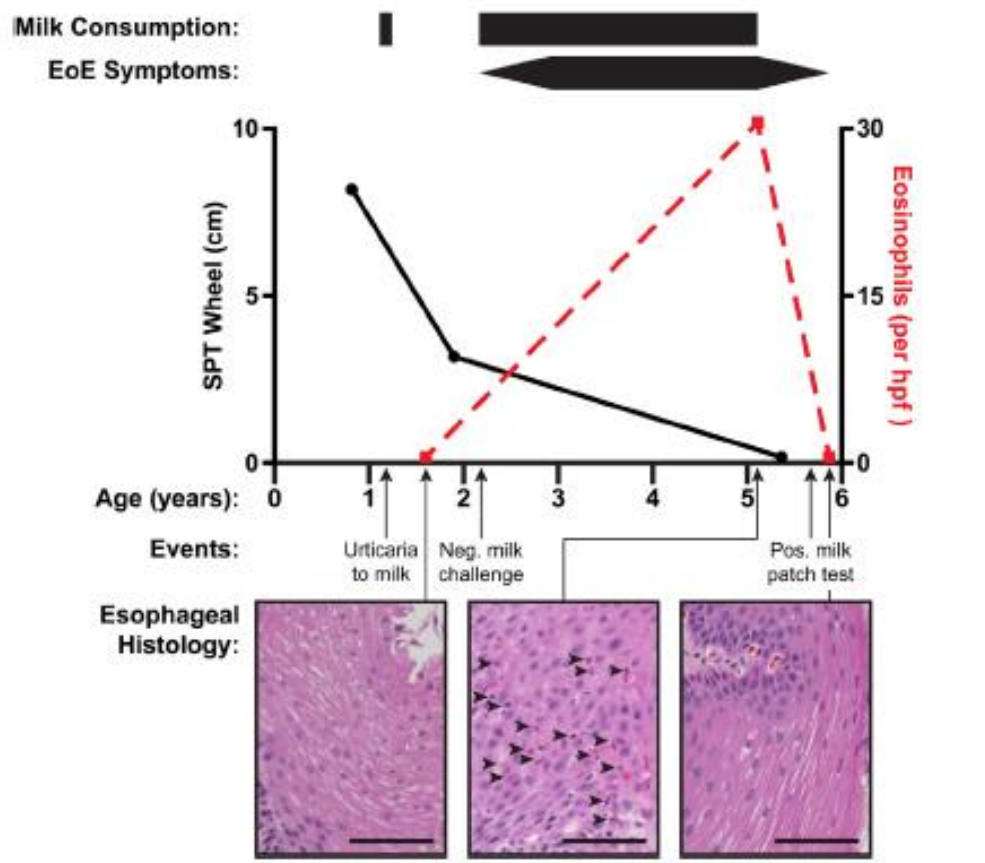
- **Large concomitant atopy up to 80% in Pediatrics and Adults**

	Number of patients with EoE	Atopy	Asthma	Allergic Rhinitis	Atopic dermatitis	IgE specific for foods	Anaphylaxis to foods
General population	NA	30%	8.5%	25%	10%	10%	0.2%
Spergel *et al.* Philadelphia	620	NA	50%	61%	21%	50	10%
Assa'ad *et al.* Cincinnati	89	79%	39%	30%	19%	75%	NA
Sugnanam *et al.* Australia	45	NA	66%	93%	55%	NA	24%
Guajardo *et al.* World registry	39	80%	38%	64%	26%	62%	23%



Hill DA 2018

Outgrowing FA and developing EoE

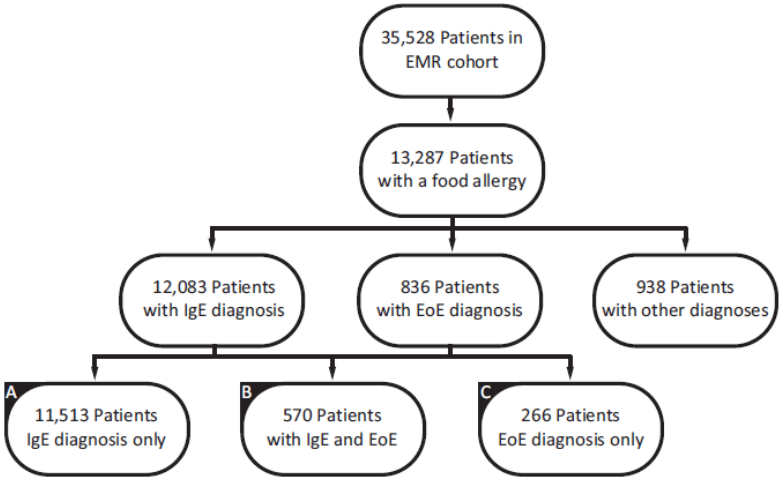


-Resolution of acute IgE-mediated allergy with development of eosinophilic esophagitis triggered by the same food

-Showed this mechanism in about 30 patients

Maggadottir SM JACI 2014

Children with IgE - FA are at risk of developing EoE



Characteristic	Odds ratio	95% CI	*P* value
Gender			
Male vs Female	2.27	1.89-2.78	<.0001
Race			
White vs Black	1.99	1.55-2.56	<.0001
Ethnicity			
Hispanic vs Non-Hispanic	0.99	0.67-1.47	ns
Specific food allergens			
Peanut	1.07	0.90-1.27	ns
Egg	2.27	1.91-2.64	<.0001
Tree nut	1.04	0.88-1.23	ns
Milk	4.19	3.52-4.97	<.0001
Shellfish	1.55	1.24-1.92	<.0001
Number of food allergies			
2 foods	2.93	2.16-4.05	<.0001
3+ foods	5.29	3.82-7.32	<.0001

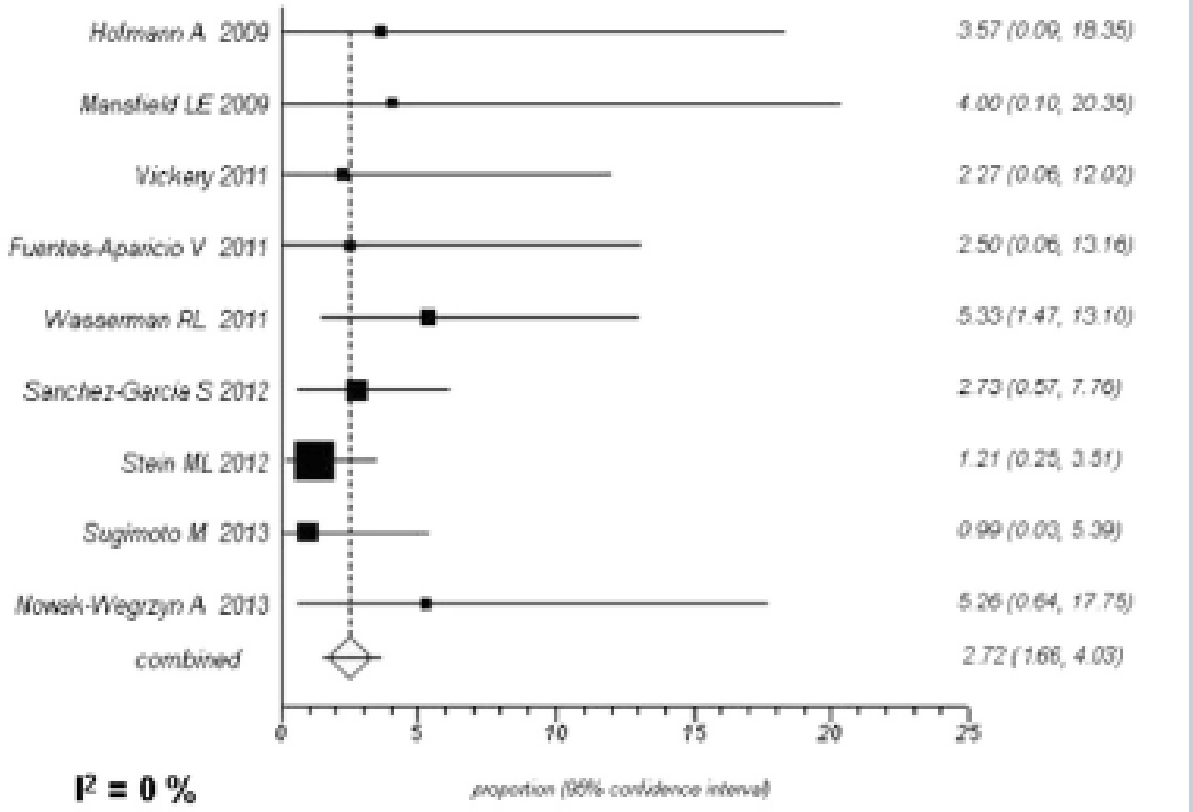
EMR, Electronic medical record; *EoE*, eosinophilic esophagitis; *ns*, nonsignificant.

Hill DA 2017

Relation between eosinophilic esophagitis and oral immunotherapy for food allergy: a systematic review with meta-analysis

Alfredo J. Lucendo, MD, PhD, FEBGH [*]; Ángel Arias, BSc, MSc [†]; and José M. Tenias, MD, PhD [†]

[*] Department of Gastroenterology, Hospital General de Tomelloso, Tomelloso, Ciudad Real, Spain
[†] Research Unit, Complejo Hospitalario La Mancha Centro, Alcázar de San Juan, Ciudad Real, Spain



Interventions	Incidence	n
EoE after immunotherapy (overall)	2.72% (1.7–4)	9
Subgroups according to quality (type of publication)		
Medium to high (full-length article)	3.51% (1.3–6.7)	3
Low (abstract)	2.5% (1.3–4)	6

Lucendo et al. Annales of Allergy 2014

Outgrowing EoE

1995-2006 (512 patients with EoE)

	Total Resolution	Outgrown Some Food Allergies
N	11	33
Age at Diagnosis (yr)	5.6 yr	4.9 yr
Follow-up (yr)	5.2yr	6.8 yr

Spergel et al. JPGN 2009

Genetic Factors in EoE

Male 3:1

General population

0.06%

Siblings

2.4%

40 times

Twin

41%

683 times

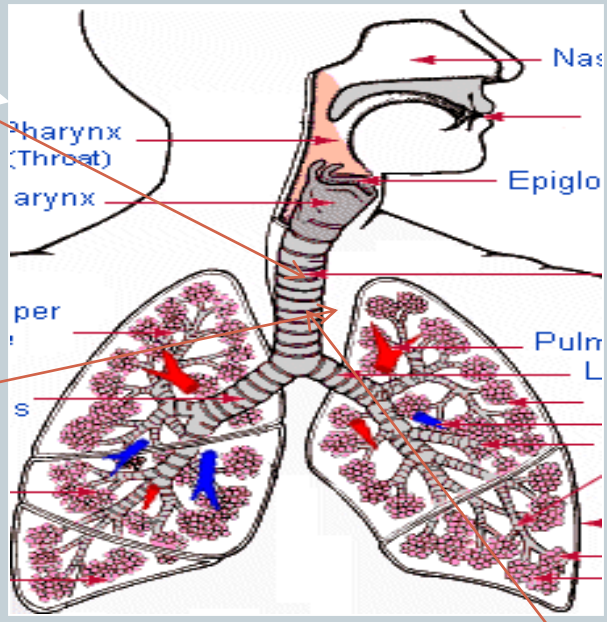
Sibling Risk in Asthma==2

Alexander et al. J Allergy Clin Immunol

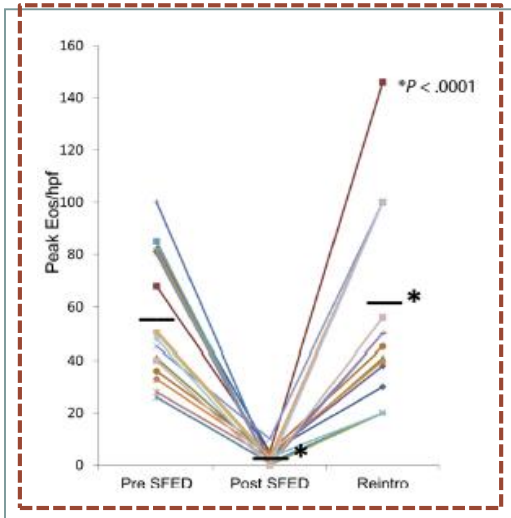
Triggers

Enviromental Allergen

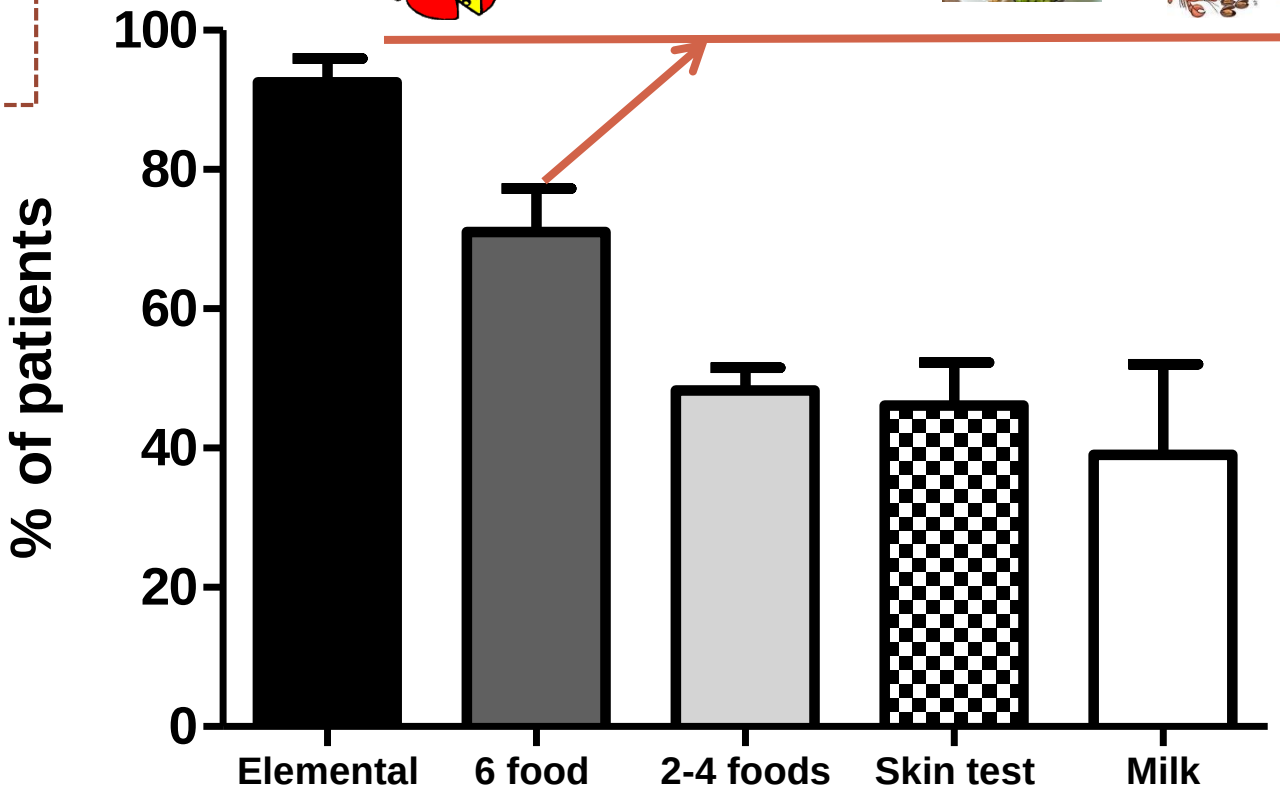
Infections



Foods



Diet Efficacy in EoE

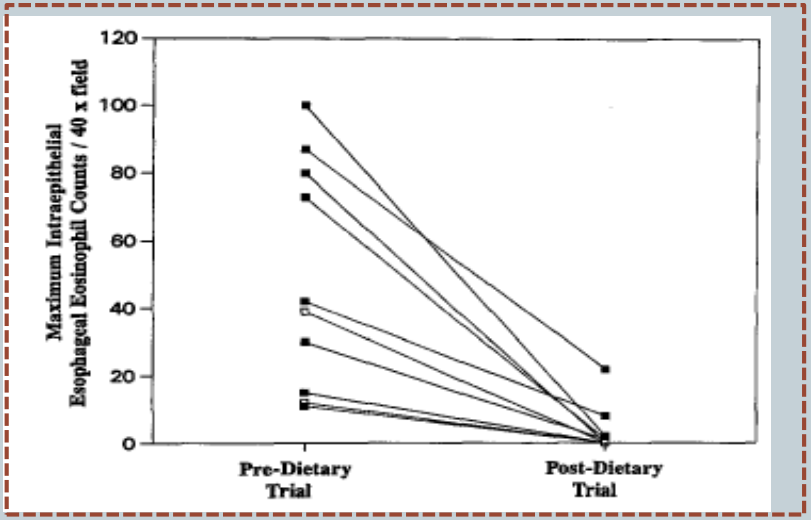


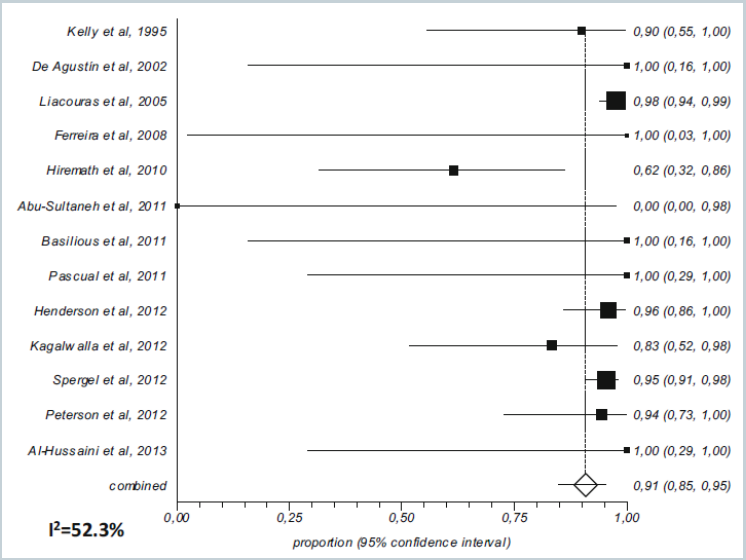
Lucendo, Curr Gastroenterol Rep.2015
Molina-Infante, JACI 2015, Molina Infante JACI 2017

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**
  - **-doesn't predict level of inflammation**
  - -Reactions may be delayed and 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% 9depending 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
Van Rhijn BD JACI 2015

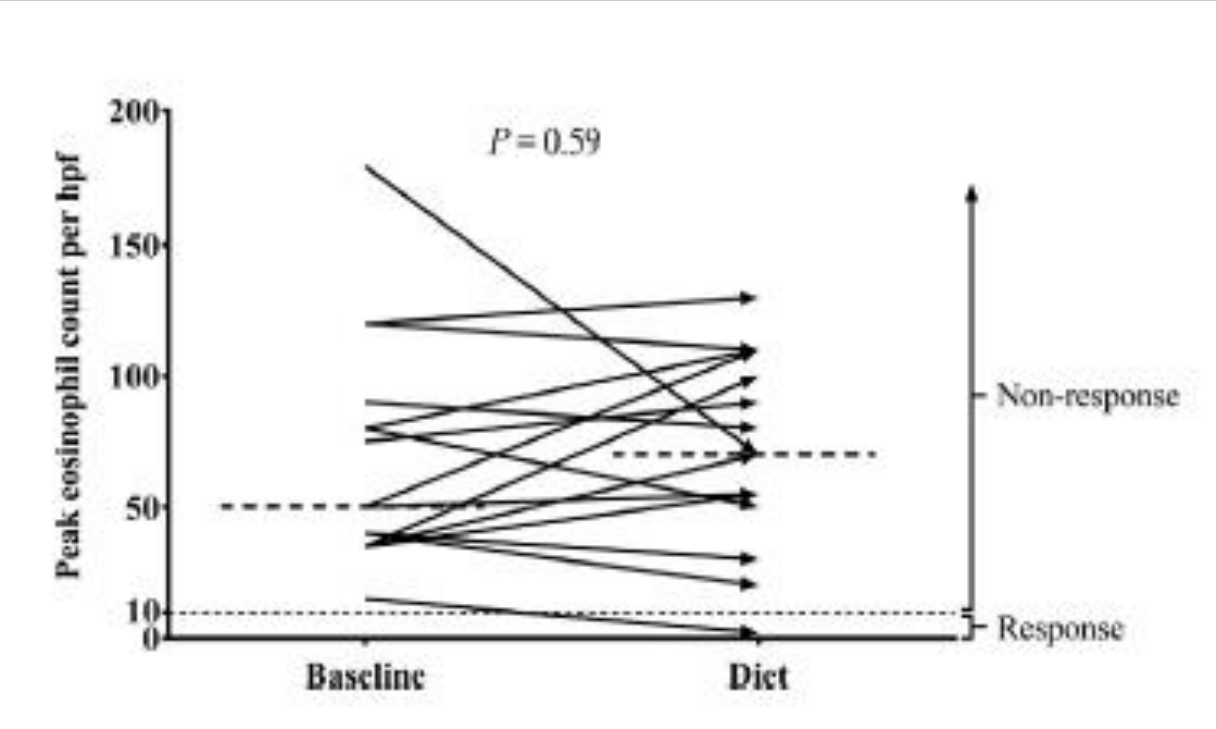
Percutaneous Testing

- Variable predictive
- Low NPV and PPVs 50-85%
- Low sensitivity
- Cross-reactivity between foods
  - Clinical significance?

	SPT			
Food	PPV	NPV	Specificity	Sensitivity
Milk (n = 46)	95.7%	57.7%	42.3%	97.6%
Egg (n = 39)	84.8%	75.4%	65.1%	90.2%
Soy (n = 28)	70.0%	68.9%	37.8%	89.5%
Wheat (n = 26)	77.8%	64.7%	18.9%	96.5%
Corn (n = 26)	57.1%	71.3%	13.8%	95.4%
Beef (n = 23)	81.8%	74.7%	30.0%	96.9%
Chicken (n = 15)	50.0%	83.3%	26.3%	93.3%
Rice (n = 14)	50.0%	85.6%	13.3%	97.5%
Potato (n = 11)	60.0%	89.9%	25.0%	97.6%
Peanut (n = 10)	77.8%	97.6%	77.8%	97.6%
Oat (n = 9)	33.3%	90.1%	10.0%	97.6%
Barley (n = 9)	42.9%	90.8%	27.3%	95.2%

NPV = negative predictive value;
PPV = positive predictive value.

IgE Microarray (CRD) fail to identify trigger foods in EoE



van Rhijn BD JACI 2015

Six Food Elimination Diet (SFED) Adults

- Food Reintroduction

- most common food triggers were

wheat (60%)

Milk (50%),

soy (10%),

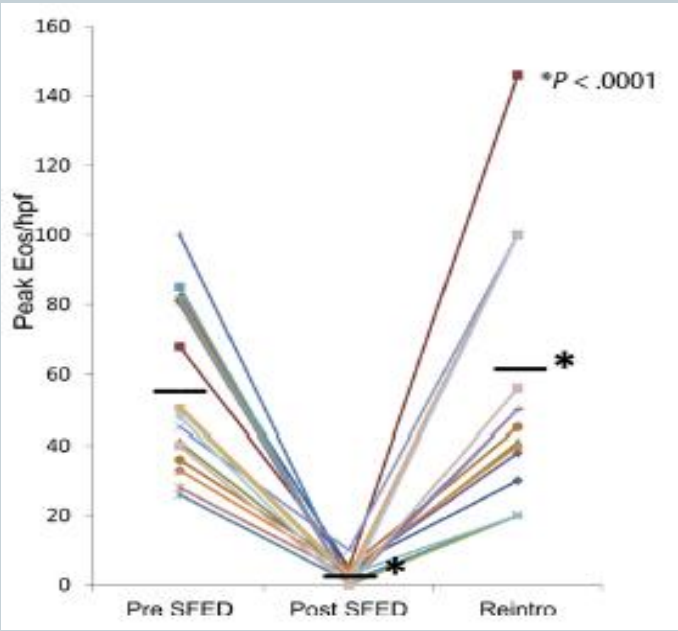
nuts (10%),

egg (5%).

seafood (0)

- Three patients had more than one food trigger

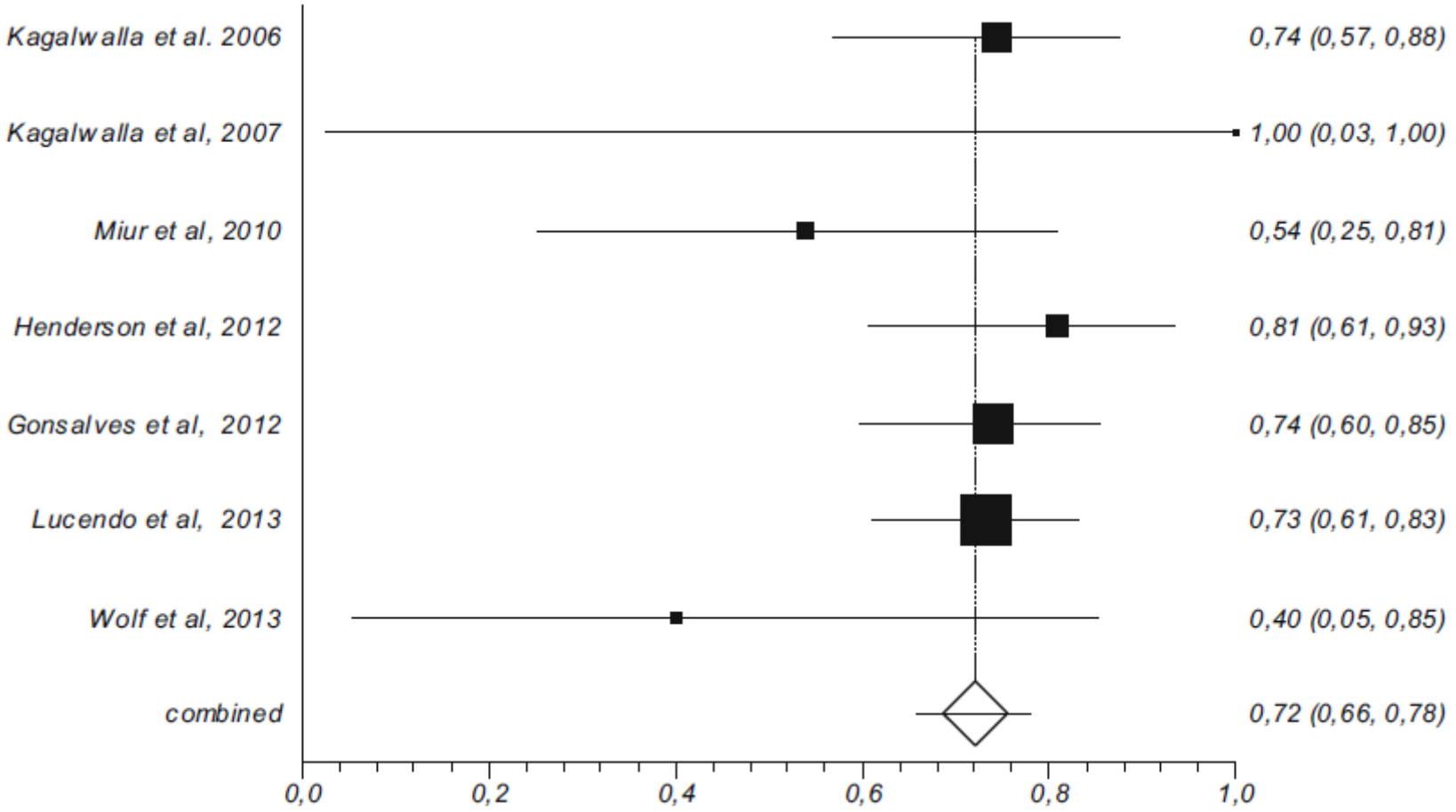
- SPT accurately predicted only 13% of causal agents, and 67% of patients who had a food trigger identified by the reintroduction process had a negative SPT to all foods



Common food allergens on skin prick test[a]	
Nuts	26/50(52%)
Wheat	10/50 (20%)
Soy	10/50 (20%)
Seafood	6/50 (12%)
Egg	6/50 (12%)
Milk	3/50 (6%)

Gonsalves *et al*. Gastroenterology 2012

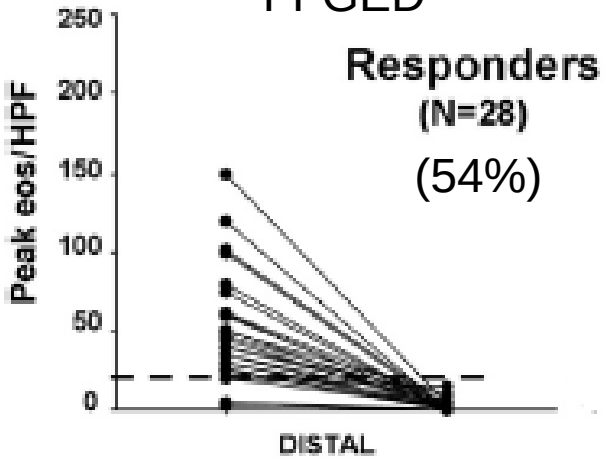
SFED and EoE



Lucendo, Curr Gastroenterol Rep.2015

Four Food Elimination Diet (Milk, wheat, egg, legumes) Adults

FFGED



SFGED after failure of FFGED

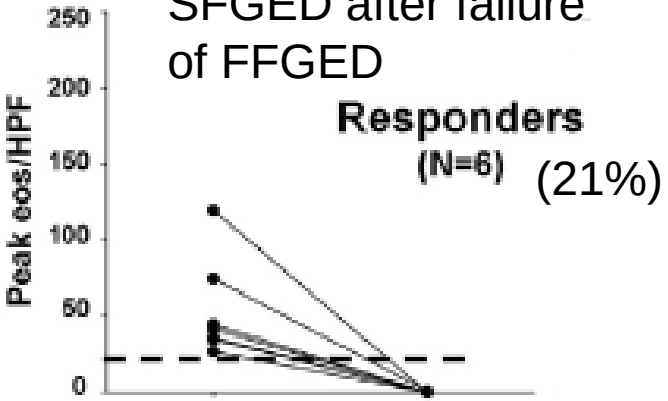
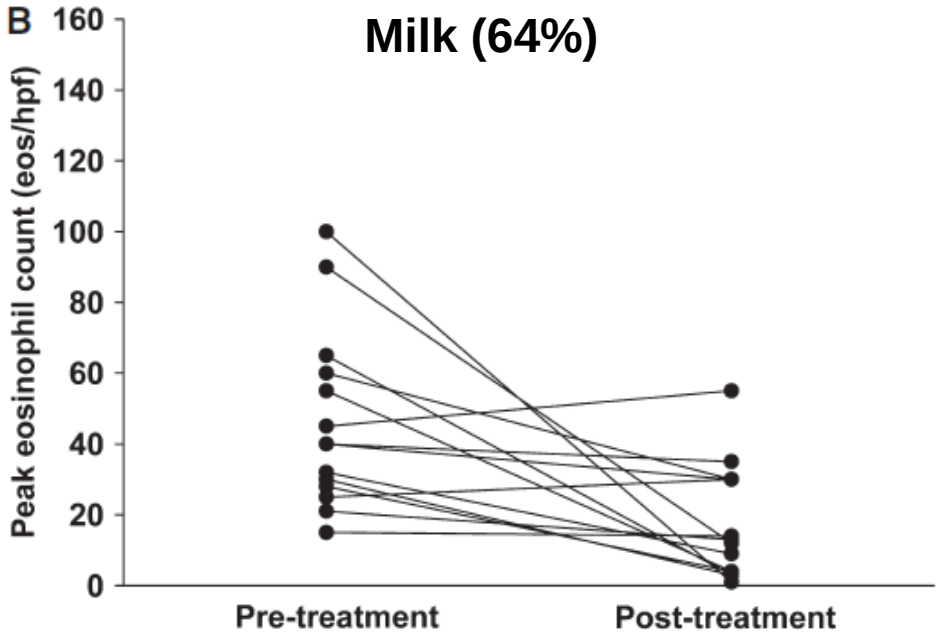


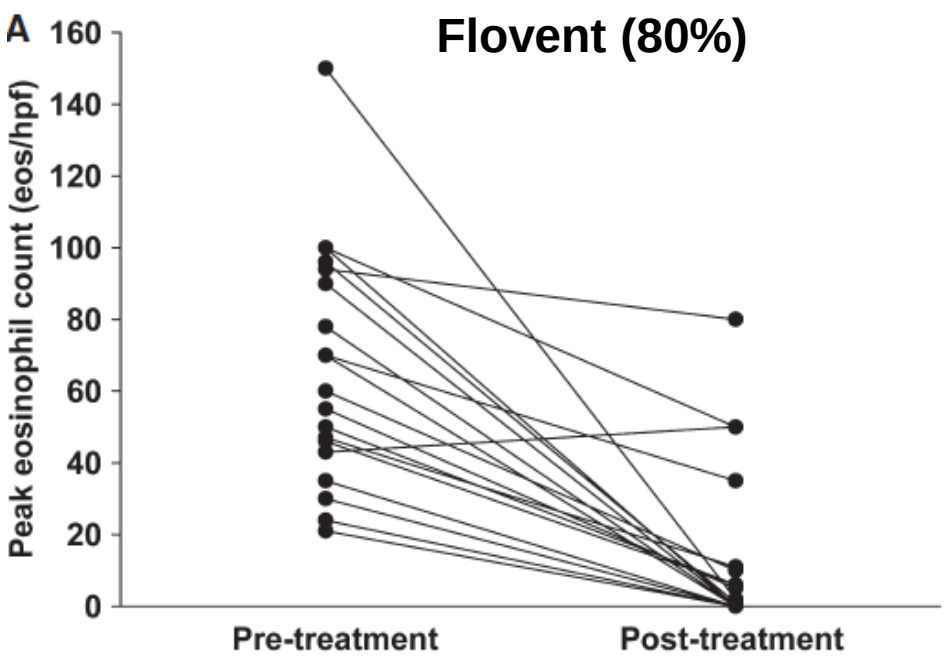
TABLE II. Food triggers identified by sequential food challenge (n = 22) after response to the FFGED

A single causative food group	10/22	45%
Milk	6/22	27%
Wheat	3/22	13%
Egg	1/22	4%
Two causative food groups	10/22	45%
Milk and egg	2/22	9%
Milk and legumes	2/22	9%
Milk and wheat	1/22	4%
Wheat and egg	3/22	13%
Egg and legumes	2/22	9%
Three or more causative food groups	0	0
No causative food group	2/22	9%

Molina-Infante, JACI 2015

One Food Elimination Diet (Milk) children

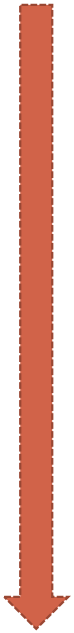




Kruszewski PG et al, Dis Esophagus 2015

6 Food Elimination diet

Step down approach



Reintroduction of 1 single food every 6-12 weeks followed by EGD

Milk elimination diet

Step up approach

Elimination of 1 single food (from the 6 food elimination pool) every 6-12 weeks followed by EGD

GOAL = Find the least restrictive diet that can control EoE

Most Common Foods in EoE

TABLE 3. *The 10 most common foods confirmed by endoscopy after allergy testing*

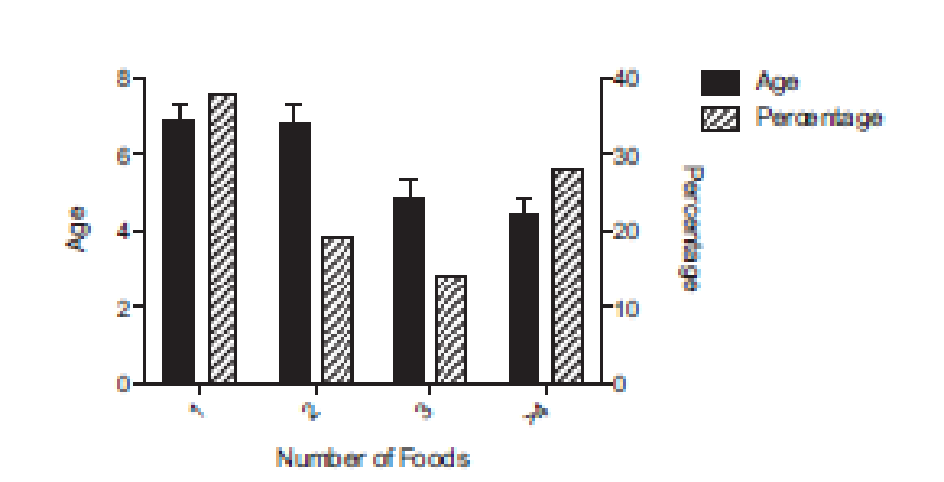
Food	Causative foods, %
Milk	17
Egg	11
Wheat	9.6
Soy	7.8
Corn	7.8
Beef	6.6
Chicken	6.1
Peanut	5.4
Potato	4.8
Rice	4.1

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)

Food	No. of subjects
Milk	78
Milk, meats*	24
Milk, egg, wheat, soy	20
Milk, soy	15
Grains*	13
Milk, egg, wheat, meats	11
Egg, wheat	10
Milk, egg	8
Milk, egg, wheat	8
Egg	8
Soy	7
Wheat	5



Younger children sensitive to more foods

Spergel et al JACI 2012

Some patient with milk induced EoE are able to tolerate baked milk

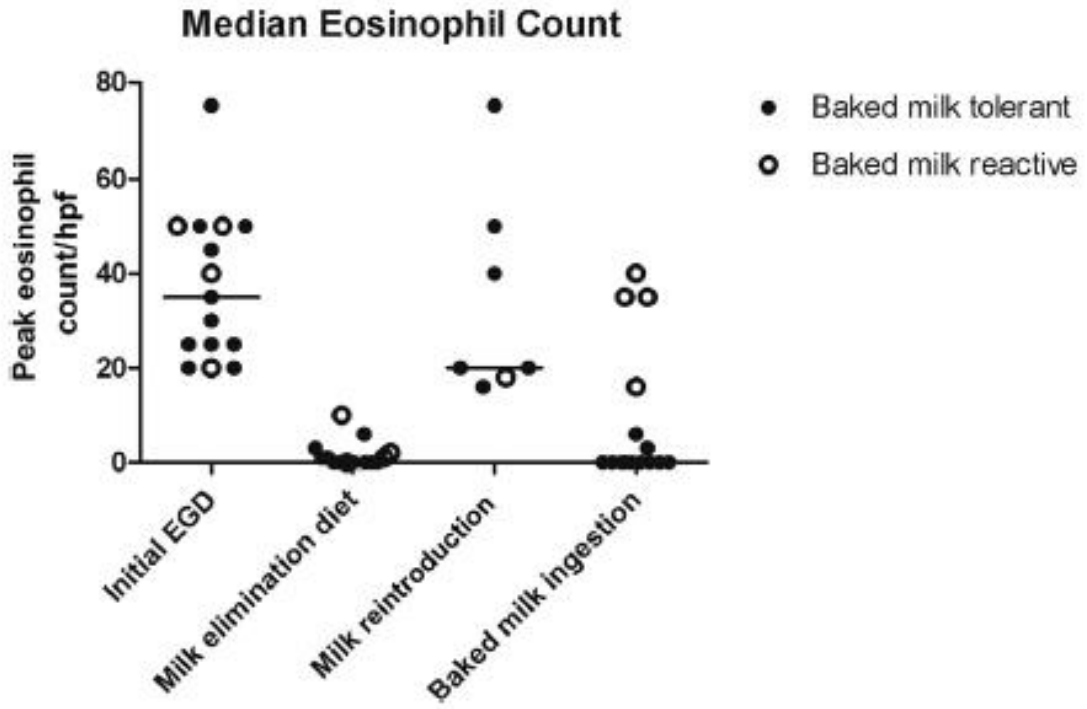


FIG E1. Median peak eosinophil count/hpf at different time points.

Leung J, JACI 2013

Baked Milk/Egg allergen

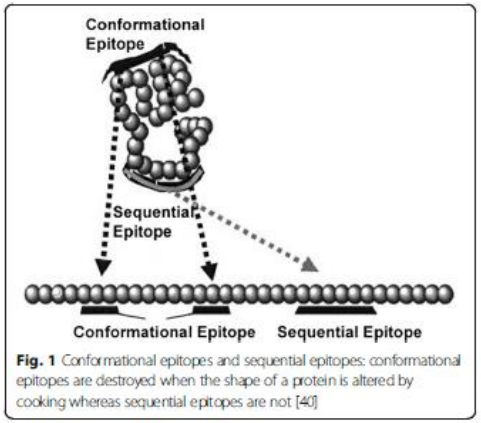
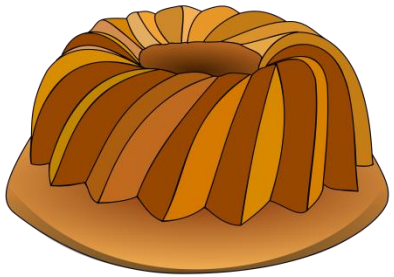


Fig. 1 Conformational epitopes and sequential epitopes: conformational epitopes are destroyed when the shape of a protein is altered by cooking whereas sequential epitopes are not [40]

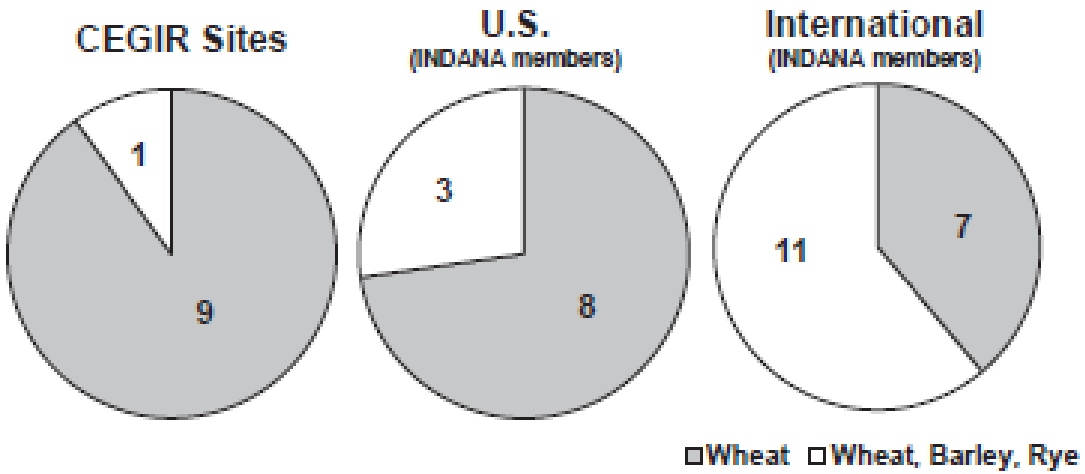
It is well known that cooking and/or processing can
- denature conformational epitopes, making them no longer recognizable by the epitope-specific IgE.
-strengthen certain protein bonds or create neoepitopes, such as when amino acids react with aldehyde or ketone groups on sugars (glycation) in enzymatic 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

Baked Milk/Egg allergen: Matrix effect



- 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

Should wheat, barley, rye, and/or gluten be avoided in a 6-food elimination diet?



Gluten-containing Grains Eliminated on 6FED for EoE

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

JACI, 2016

How food can induce Eosinophilic-Th2 inflammation in EoE?

Food allergens, other trigg

Eosinophilic- Th2 Inflammation

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% effectiveAfter administration of topical steroids no food or drink is allowed for 30 minutes

- Accepted 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

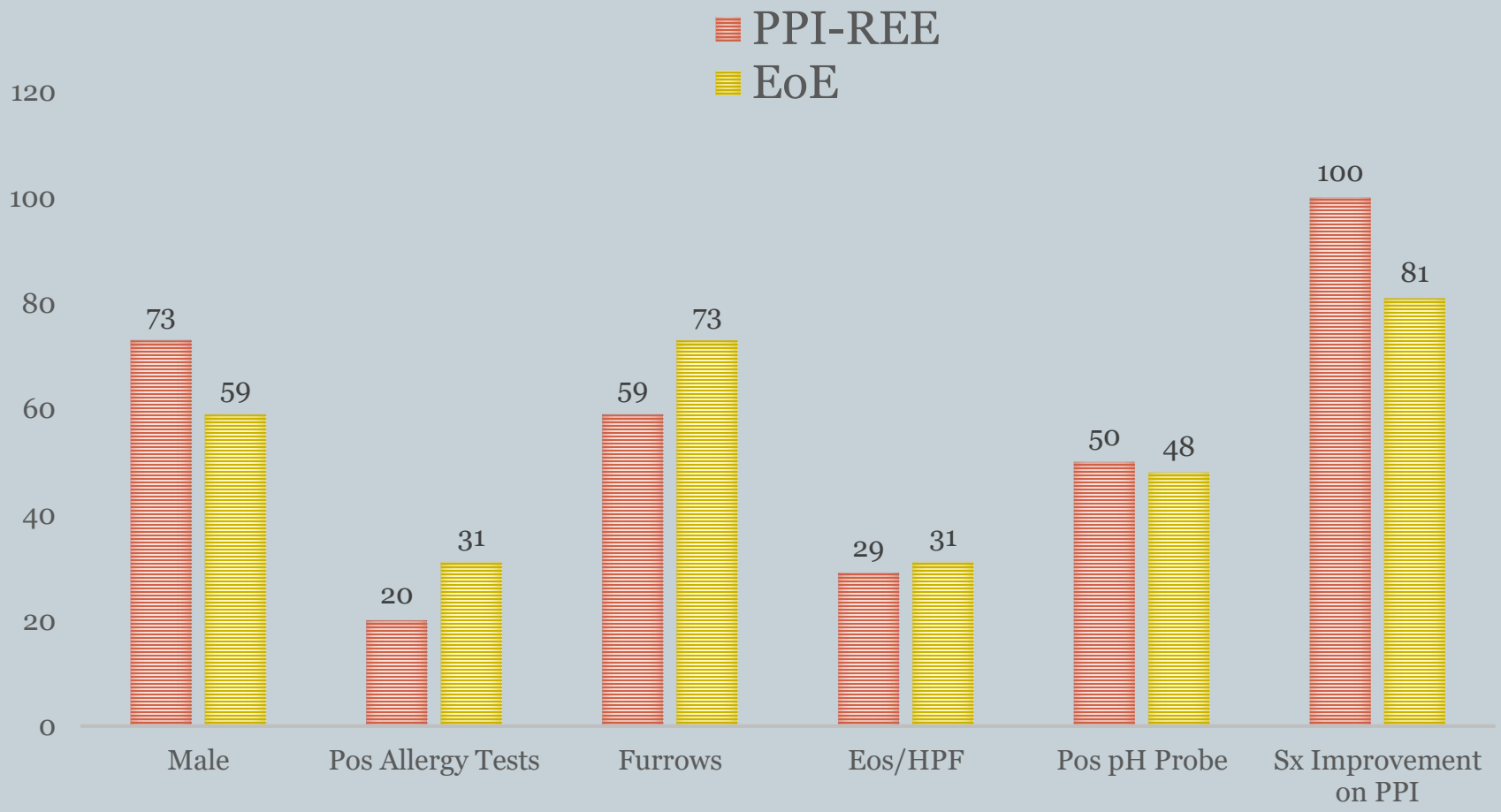
- Aerosolized **fluticasone propionate** from a metered dose inhaler (no spacer) 110 mcgX 2 puffs twice daily for children year< 8 years of age;220 mcg X 2 puffs twice daily for children year>8 years of age
- **Budesonide** is delivered as viscous budesonide, is made by mixing each 1 mg Pulmicort RespuleTM with 10 g (10 packets) of sucralose (SplendaTM) to create a volume of approximately 8mL .0.5-2mg in 1 or 2 dived doses

Liacouras *et al*. Clin Gastroenterol Hepatol 2005
Furuta *et al*. Gastroenterology 2007
Cianferoni a et al 2015

PPI-Responsive EOE PPI-REE Estimates

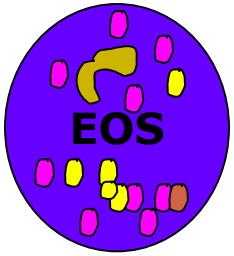
Author	Year	Population	Design	# Patients	%
Dranove	2009	Pediatric	Retro	43	40
Sayej	2009	Pediatric	Retro	36	39
Monte-Infante	2011	Adult	Prospective	35	74
Peterson	2010	Adult	RCT	12	33
Moawad	2011	Adult	RCT	20	35
Dellon	2013	Adult	Prospective	65	37
Schroeder	2013	Adult	Retro	7	71

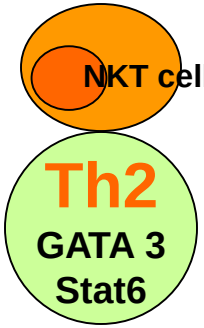
PPI-REE vs EoE

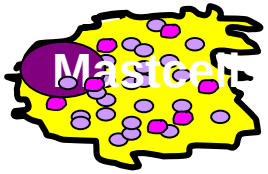


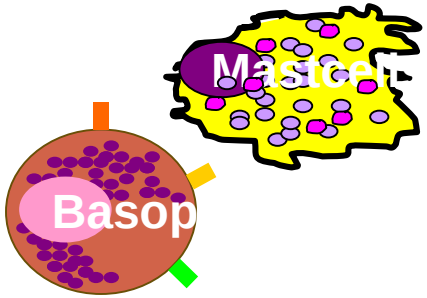
Dranove et al J Ped 2009

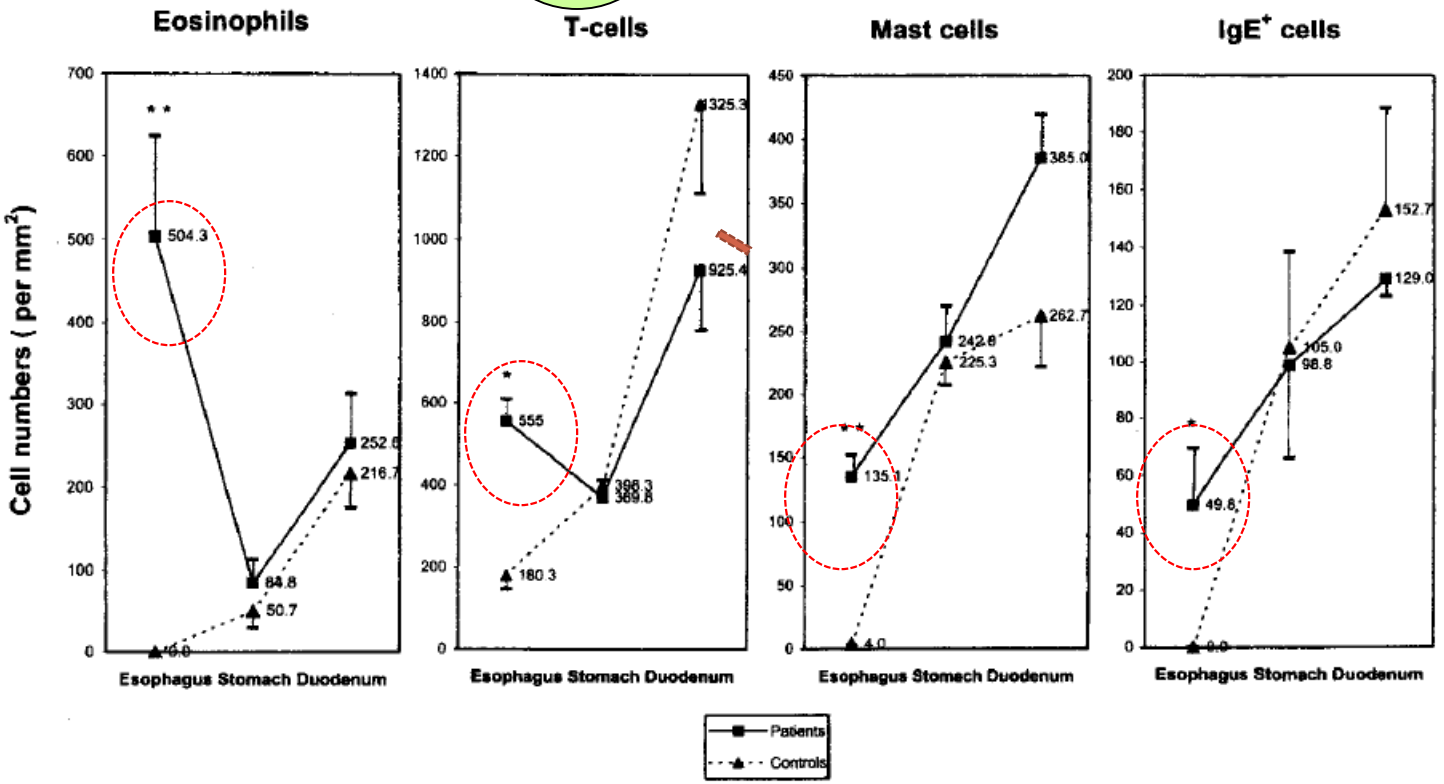
Which are the cells that produce Th2 cytokines in EoE?











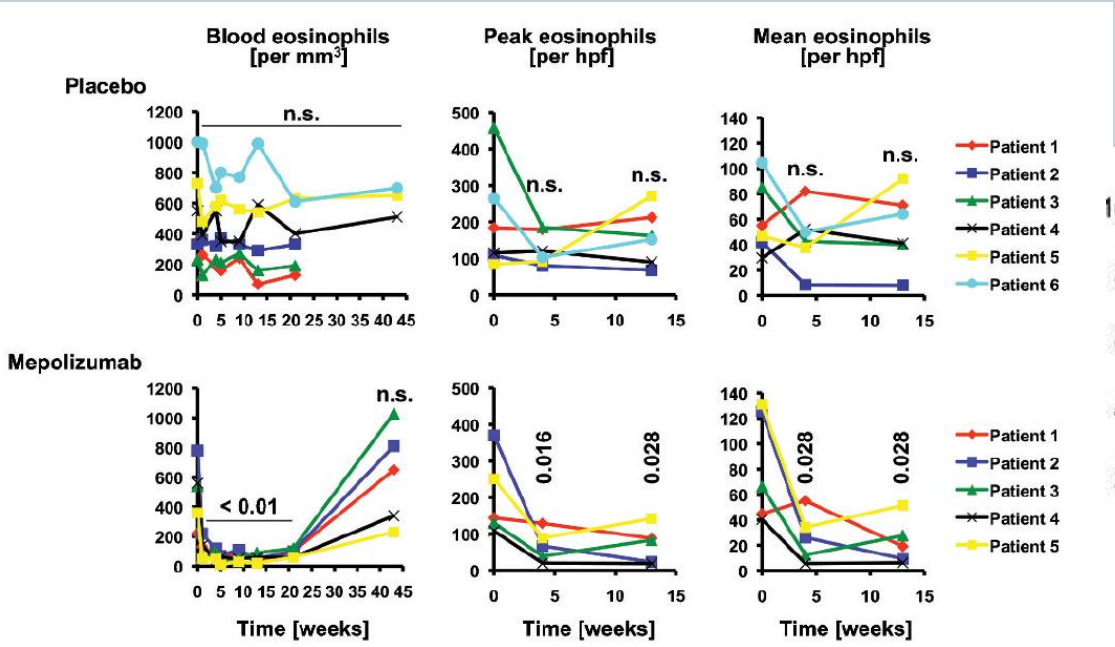
(Strauman AJ Allergy Clin Immunol 2001;108:954-61.)

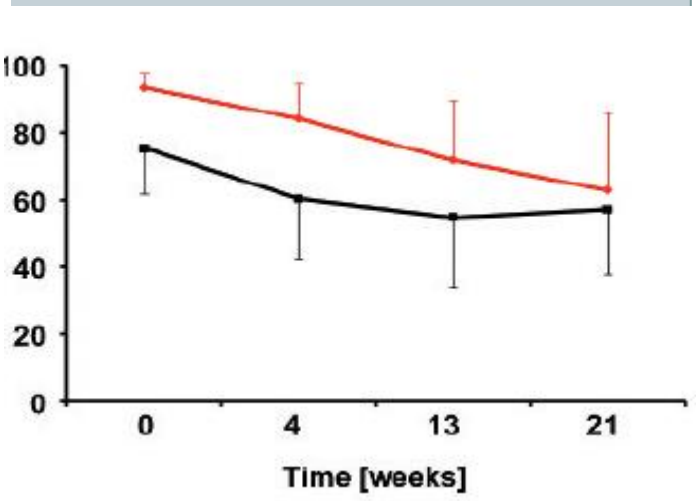
Cytokines in EoE pathogenesis

Publication	Human Cytokine Finding
Straumann et al JACI 2002	•Increased expression of IL-5 •TNF-a was increased in epithelial cells
Straumann et al. Inflamm Bowel Dis, 2004	Eosinophils express "activation" markers: 60% express **IL-13 and IL-4** 41% of intestinal eosinophils express IL-13 at baseline, circulating eosinophils do not express IL-4 or -13
Gupta et al. Am J Gastro 2006	No increase in cysLT
Blanchard et al. J Clin Invest 2006	50-fold increase in **Eotaxin-3** in biopsy
Aceves et al. JACI 2007	Esophageal remodeling with increased TGFb, phospho-Smad2/3, VCAM
Battacharya et al, Hum Pathol 2007	Increased Eotaxin-3 in archived biopsies
Blanchard C et al JACI 2011	Increased **IL-13, IL-4, IL-5 IL-15** in esophageal bx of patients with active EoE
Zhou H et al Gastroenterology 2010	Eosinophialia in Esophageal bx correlates with IL-15 levels
Rothenberg ME, Spergel JM, Nat Genet. 2010	Increased TSLP in esophageal bx of patients with active EoE

Anti-IL-5 EoE

•Anti- IL-5 (mepolizumab/reslizumab) reduces Eosinophilic infiltration in humans but has no significant effects on dyshpagia





Spergel et al 2011 abstract
Strauman A Gut 2010

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY

EAACI

Allergy

REVIEW ARTICLE

Eosinophilic esophagitis is characterized by a non-IgE-mediated food hypersensitivity§

D. Simon[1,*], A. Cianferoni[2,3,*], J. M. Spergel[2,3], S. Aceves[4], M. Holbreich[5], C. Venter[6,7], M. E. Rothenberg[6], I. Terreehorst[8], A. Muraro[9], A. J. Lucendo[10], A. Schoepfer[11], A. Straumann[12] & H.-U. Simon[13]

:

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 .

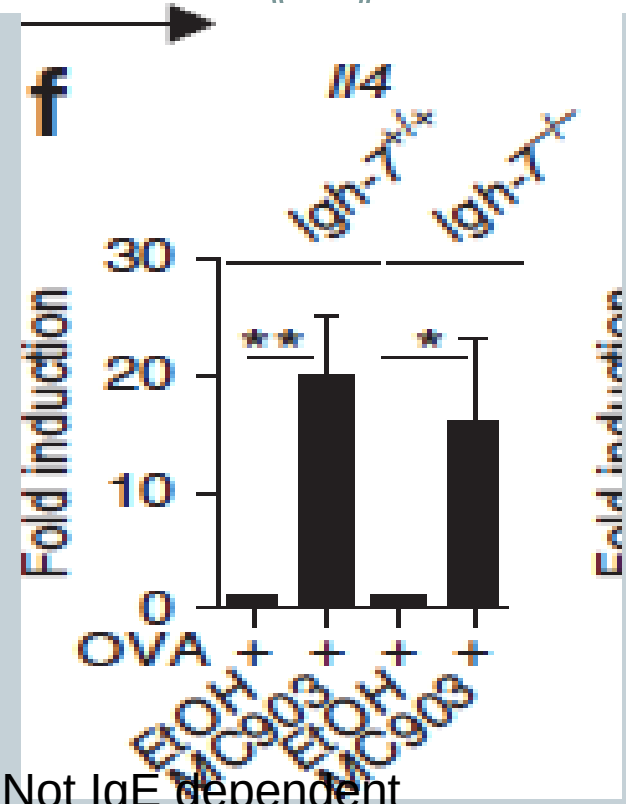
3) Oral immunotherapy, which has been used successfully in IgE-mediated food allergy, is associated with an increased risk of developing EoE (e.g. in 2 to 10% of treated patients)

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

In animal model EoE is Non IgE mediated food allergy



Not IgE dependent

Noti et al, Nat. Medicine 2013

EoE ≠ IgE Mediated Disease

	Omalizumab	Placebo
Number of subjects	16	14
Eosinophils/high-power field, before treatment	41 ± 17	37 ± 9
Eosinophils/high-power field, after treatment	39 ± 15	33 ± 12
Change in mean eosinophil content	-1.3	-4.2
Dysphagia score before treatment	4.0 ± 0.7	5.5 ± 0.5
Dysphagia score after treatment	2.8 ± 1.0	3.8 ± 0.6
Change in dysphagia score after treatment	-1.2[a]	-1.7[a]

In a double blind placebo control trial Omalizumab Has No Effect on Eosinophil Content or ,Relative to Placebo Controls, Symptoms

Table 2. Esophageal Mucosal Tissue IgG4 Is Strikingly and Specifically Increased

	Normal controls	Eosinophilic esophagitis	Eosinophilic esophagitis/control ratio
IgM, mg/g protein	0.16 ± 0.07	0.38 ± 0.21	2.4 (0.91–5.5)
IgA, mg/g protein	0.32 ± 0.15	0.51 ± 0.18	1.6 (0.84–3.5)
IgG1, mg/g protein	2.2 ± 0.73	3.1 ± 1.1	1.4 (0.78–2.4)
IgG2, mg/g protein	0.76 ± 0.14	1.4 ± 0.64	1.8 (0.88–2.9)
IgG3, mg/g protein	0.10 ± 0.05	0.15 ± 0.092	1.5 (0.48–3.7)
IgG4, mg/g protein	0.029 ± 0.013	1.3 ± 0.99[a]	45 (7.5–109)
IgG4, % total IgG	0.92 ± 0.43	17 ± 7.7[a]	18 (8.4–40)

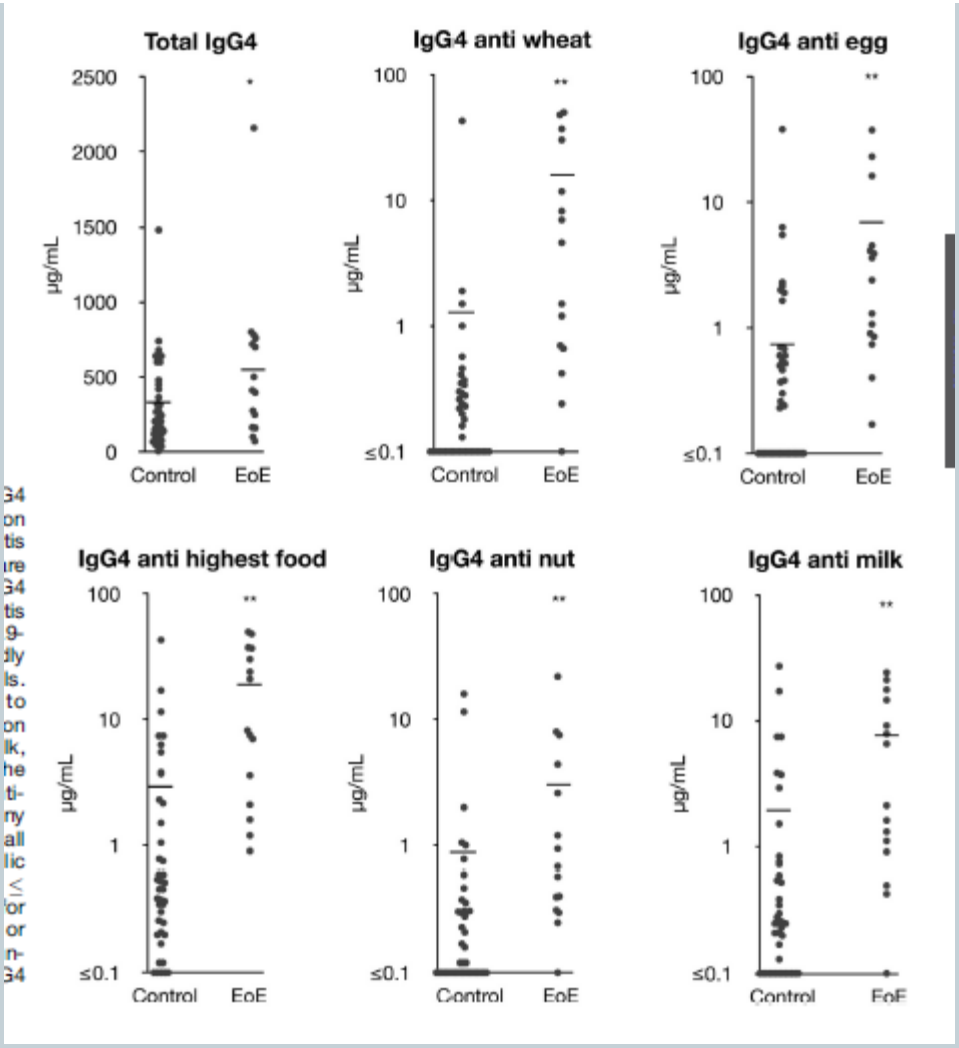
Clyaton F 2014

EoE ≠ IgE Mediated Disease

Immunoglobulin, median (IQR)	Control subjects	Subjects with EoE	EoE/control ratio	*P* value[b]
IgE	19.5 (18.8-20.4)	18.7 (18.2-19.1)	1.0	.0620
IgA	0.9 (0.5-1.4)	2.0 (1.8-4.6)	2.2	<.0001
IgM	0.9 (0.6-1.5)	2.3 (0.9-3.9)	2.6	.0102
IgG1	9.0 (6.6-10.2)	13.4 (9.5-18.4)	1.5	.0132
IgG2	8.1 (2.7-11.2)	16.2 (9.7-32.6)	2.0	.0122
IgG3	0.6 (0.5-1.0)	2.2 (1.3-5.1)	3.7	<.0001
IgG4	0.2 (0.1-0.9)	4.2 (1.0-13.1)	21.0	<.0001
IgG4, % total IgG	4.5% (1.8-9.3)	18.0% (6.5-23.5)	4.0	.0122

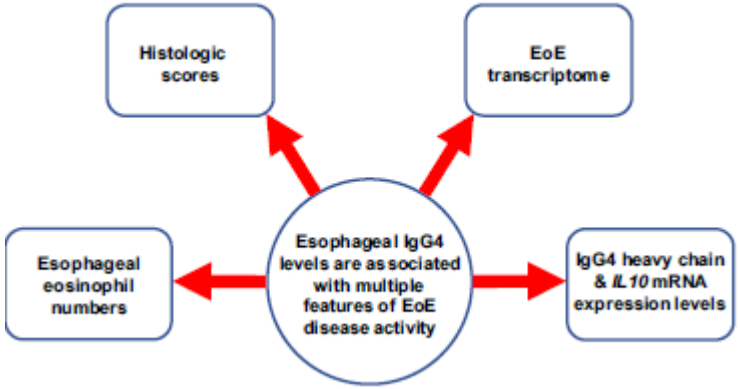
Rosenberg CE, Allergy 2018

IgG4 specific for some foods maybe important, but their clinical value is to be determined



Clayton et al Gastroenterology 2014

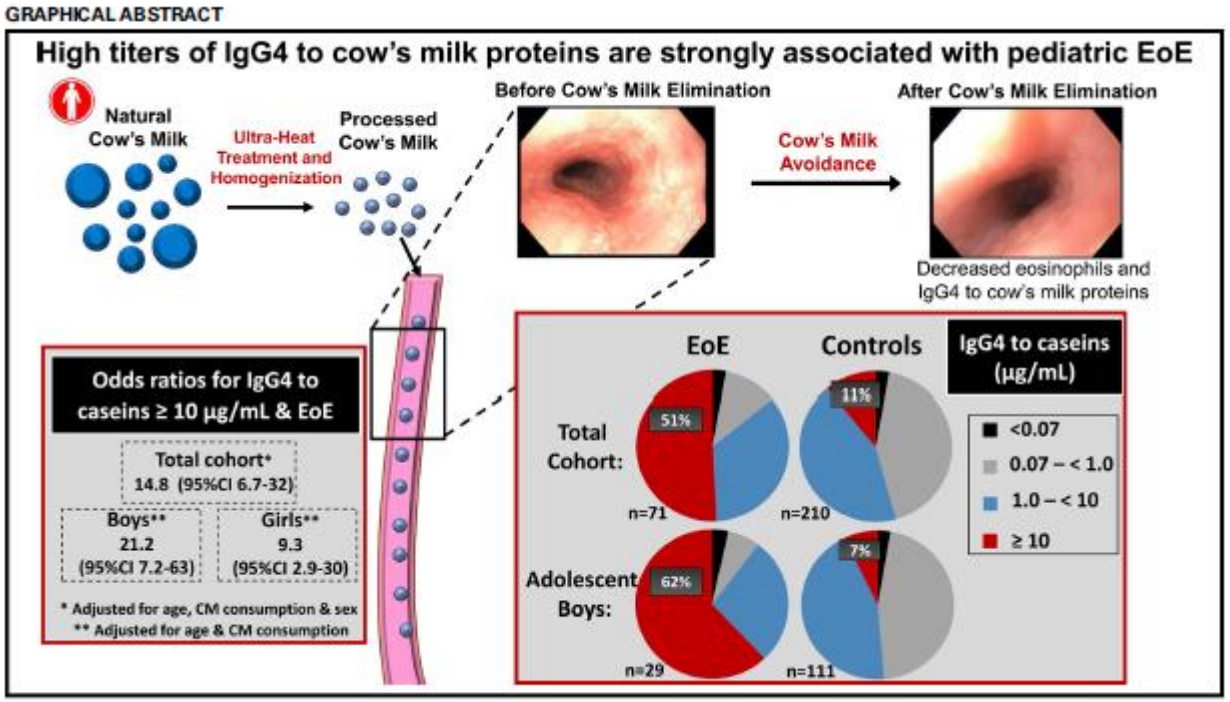
IgG4 AND EOE



• Esophageal IgG4 levels are increased in patients with EoE compared with control individuals and strongly correlate with
esophageal eosinophil numbers and multiple features of histological grade and stage scores.
• Esophageal IgG4 protein levels correlate with multiple components of the disease as assessed by transcriptome profiling, including IL4, IL13 and IL10 mRNA expression levels.
• IgG4 heavy chain mRNA expression is proportional to IgG4 protein levels and IL10 mRNA expression levels in the esophagus of patients with EoE..

Rosenberg CE, Allergy 2018

IgG4 to cow's milk protein in EoE



Schuyler AJ et al JACI 2018

IgG4 to cow's milk protein in EoE

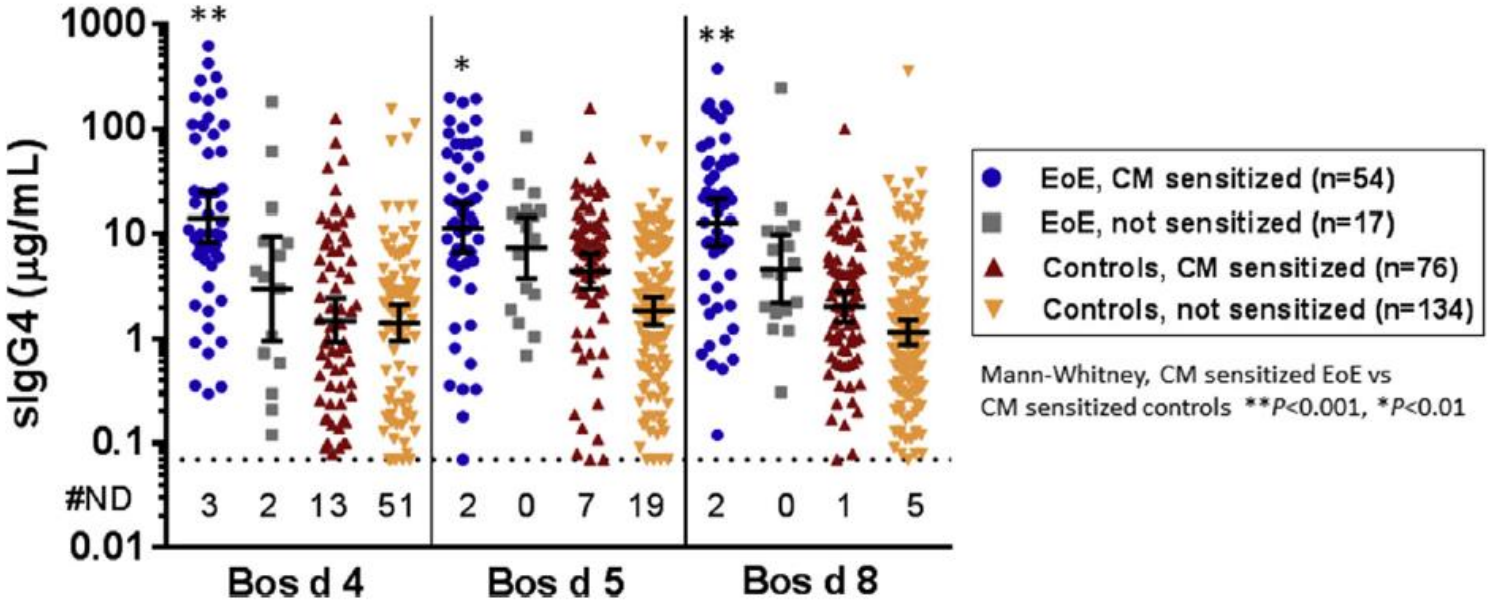


FIG 2. $sIgG_4$ levels (geometric mean [95% CI]) to Bos d 4, Bos d 5, and Bos d 8 in 71 patients with EoE and 210 control subjects with or without CM sensitization. *Values below the dotted line* indicate the number not detectable *(#ND)* and were excluded from calculation of the geometric mean. Statistical analysis was performed with the Mann-Whitney test.

Schuyler AJ et al JACI 2018

IgG4 to cow's milk protein in EoE

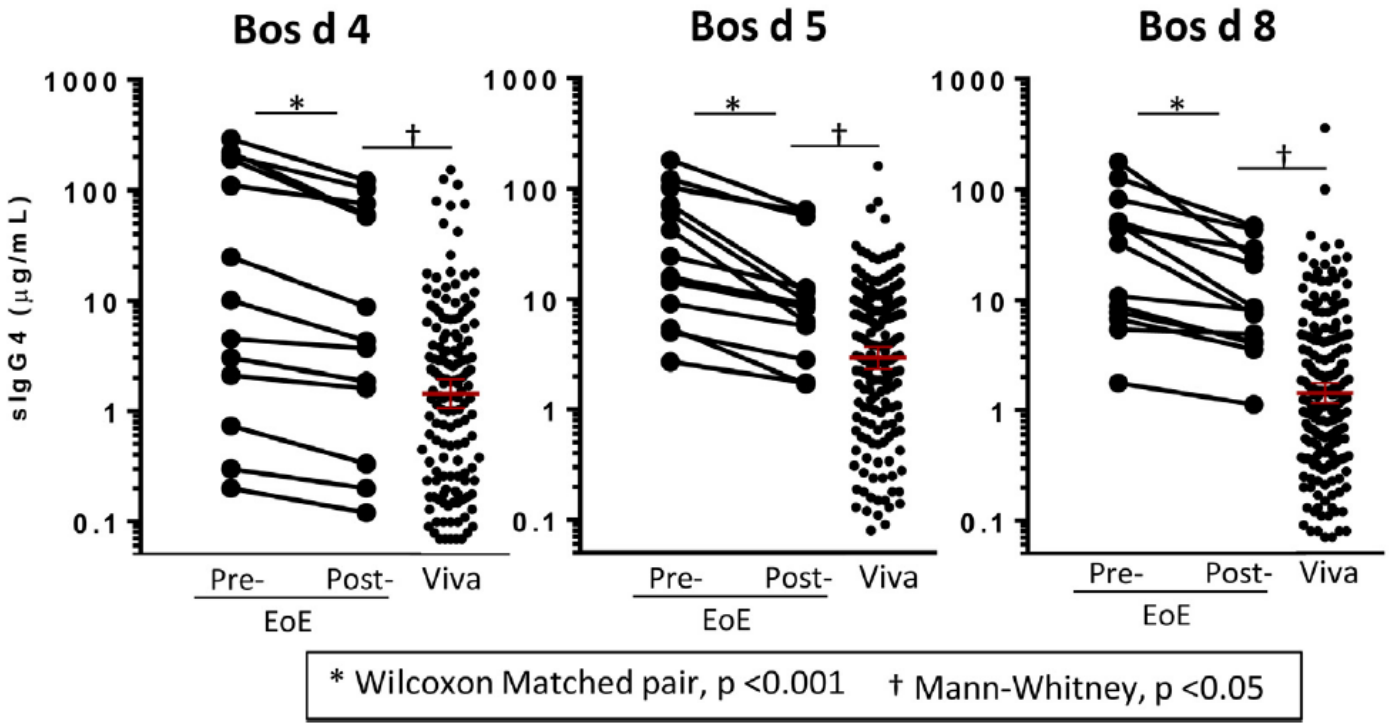


FIG 4. sIgG$_4$ levels to Bos d 4, Bos d 5, and Bos d 8 in patients with EoE before and after a 6- to 8-week CM elimination diet (n = 13) compared with baseline levels in control subjects (n = 210). Statistical analysis was performed with the Wilcoxon matched pair test and the Mann-Whitney test.

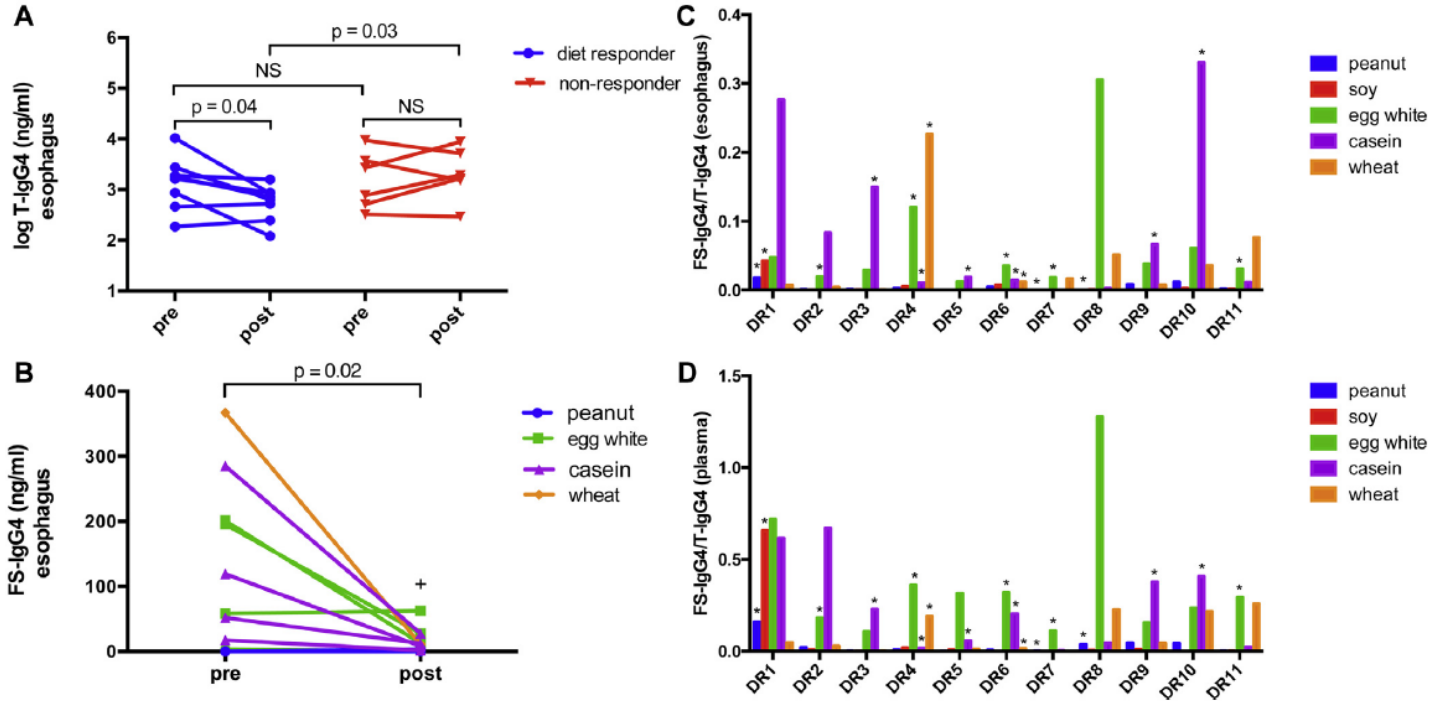
Schuyler AJ et al JACI 2018

Can sIgG$_4$ levels to CM be useful as a biomarker for the diagnosis or monitoring of EoE?.

- Need prospective investigation,
- Large overlapping between normal and EoE
- the difference in ORs between girls and boys reported here suggests that this question should be addressed with consideration to sex.

Schuyler AJ et al JACI 2018

IgG4 and EoE Food allergy in adults



Wright et al. 2016

IgG4 Are they pathogenetic?

- $sIgG_4$ is not sufficient as to cause EoE is supported by the fact that 10% of control subjects also had high-titer $sIgG_4$ to CM proteins
- CM $sIgG_4$ is an epiphenomenon in patients with EoE, perhaps related to an aberrant T_H2 or regulatory T-cell response.
- A pathogenic role for IgG_4 cannot be dismissed
- IgG_4 could be pathogenic by forming extracellular immune complexes, due to $sIgG_4$ antibody levels largely restricted to a single allergen (group) in presence of high concentrations of allergen in the tissue before Fab arm exchange occurred. Indeed $sIgG_4$ levels to CM proteins contributed more than 10% of total IgG_4 in 35% of our patients with EoE.
- Taken together, the question of whether high-titer $sIgG_4$ is an epiphenomenon or is mechanistically involved in the inflammation seen in patients with EoE is an important area for future inquiry.
- B-cell class-switch recombination (CSR) to IgG4 like the IgE one is induced by IL-4 and IL-13. IgG4 has also been associated with high levels of IL-10 secreted from regulatory B and T cells
- Patients with EoE have increased levels of TGF-b1 localized to eosinophils and mast cells in the esophagus. TGF-b1 can suppress IgE CSR and also promote mast cell accumulation and eosinophil survival.
- T cell play a central role in thus upstream defects in T-cell activation or development could contribute to both the pathology and IgG4 production in patients with EoE

The IgE: IgG4 conundrum

In Eosinophilic Esophagitis:

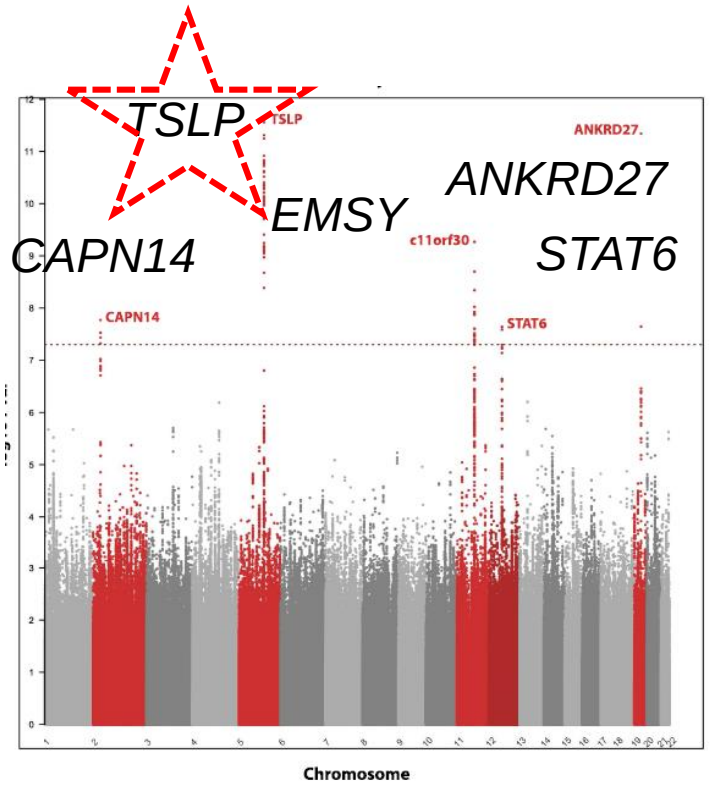
1. IgG4 to food proteins is increased – but role in pathophysiology of disease is unclear

Genetic Studies EoE

- Candidate Gene
- Genomic Wide Association Studies

TSLP SNP	5q.22.1
TSLP-R SNP	Xp22.3; Yp11.3
Eotaxin 3	7q11.23
CAPN14	2q.23
EMSY	11q13.5

nature genetics



Common variants at 5q22 associate with pediatric eosinophilic esophagitis

Marc E Rothenberg[1,11], Jonathan M Spergel[2,3,11], Joseph D Sherrill[1,11], Kiran Annaiah[4,11], Lisa J Martin[5,11], Antonella Cianferoni[2,3], Laura Gober[2], Cecilia Kim[4], Joseph Glessner[4], Edward Frackelton[4], Kelly Thomas[4], Carine Blanchard[1], Chris Liacouras[3,6], Ritu Verma[3,6], Seema Aceves[7], Margaret H Collins[8], Terri Brown-Whitehorn[2,3], Phil E Putnam[9], James P Franciosi[9], Rosetta M Chiavacci[4], Struan F A Grant[3,4,10], J Pablo Abonia[1], Patrick M A Sleiman[4] & Hakon Hakonarson[3,4,10]

nature genetics

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

Leah C Kottyan[1–3,13], Benjamin P Davis[3,13], Joseph D Sherrill[3], Kan Liu[3], Mark Rochman[3], Kenneth Kaufman[1,2], Matthew T Weirauch[1,4], Samuel Vaughn[1], Sara Lazaro[1,2], Andrew M Rupert[4], Mojtaba Kohram[4], Emily M Stucke[3], Katherine A Kemme[3], Albert Magnusen[1,2], Hua He[5], Phillip Dexheimer[4], Mirna Chehade[6], Robert A Wood[7], Robbie D Pesek[8], Brian P Vickery[9], David M Fleischer[10], Robert Lindbad[11], Hugh A Sampson[6], Vincent A Mukkada[12], Phil E Putnam[12], J Pablo Abonia[3], Lisa J Martin[5], John B Harley[1,2,14] & Marc E Rothenberg[3,14]

ARTICLE

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GWAS identifies four novel eosinophilic esophagitis loci

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

nature medicine

Thymic stromal lymphopoietin–elicited basophil responses promote eosinophilic esophagitis

Mario Noti[1,2,27], Elia D Tait Wojno[1,2,27], Brian S Kim[1–3], Mark C Siracusa[1,2], Paul R Giacomin[1,2,4], Meera G Nair[1,2,5], Alain J Benitez[6], Kathryn R Ruymann[7], Amanda B Muir[6], David A Hill[1,2,7], Kudakwashe R Chikwava[8], Amin E Moghaddam[9], Quentin J Sattentau[9], Aneesh Alex[10–12], Chao Zhou[10–12], Jennifer H Yearley[13], Paul Menard-Katcher[14], Masato Kubo[15,16], Kazushige Obata-Ninomiya[17,18], Hajime Karasuyama[17,18], Michael R Comeau[19], Terri Brown-Whitehorn[7], Rene de Waal Malefyt[20], Patrick M Sleiman[21–23], Hakon Hakonarson[21–23], Antonella Cianferoni[7], Gary W Falk[14,24,25], Mei-Lun Wang[6,24,25], Jonathan M Spergel[2,7,24,25] & David Artis[1,2,24–26]

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.
- TSLP gene variant is associated with asthma and airway hyper-responsiveness. J. Allergy Clin. Immunol. 124, 222–229 (2009)

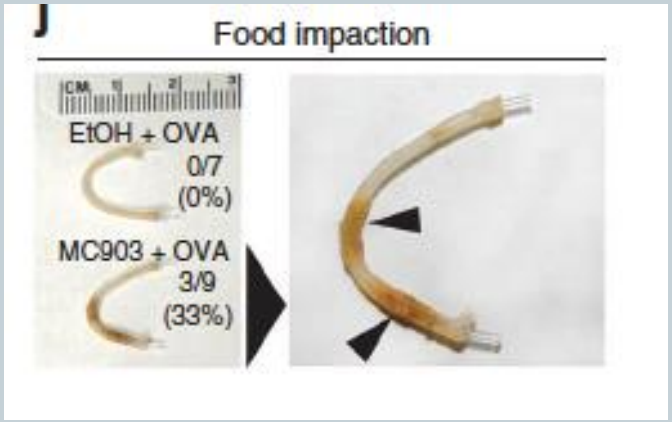
Geha Ann N Y Acad Sci. 2010 Jan;1183:13-24.

nature medicine

Thymic stromal lymphopoietin–elicited basophil responses promote eosinophilic esophagitis

Mario Noti[1,2,27], Elia D Tait Wojno[1,2,27], Brian S Kim[1–3], Mark C Siracusa[1,2], Paul R Giacomin[1,2,4], Meera G Nair[1,2,5], Alain J Benitez[6], Kathryn R Ruymann[7], Amanda B Muir[6], David A Hill[1,2,7], Kudakwashe R Chikwava[8], Amin E Moghaddam[9], Quentin J Sattentau[9], Aneesh Alex[10–12], Chao Zhou[10–12], Jennifer H Yearley[13], Paul Menard-Katcher[14], Masato Kubo[15,16], Kazushige Obata-Ninomiya[17,18], Hajime Karasuyama[17,18], Michael R Comeau[19], Terri Brown-Whitehorn[7], Rene de Waal Malefyt[20], Patrick M Sleiman[21–23], Hakon Hakonarson[21–23], Antonella Cianferoni[7], Gary W Falk[14,24,25], Mei-Lun Wang[6,24,25], Jonathan M Spergel[2,7,24,25] & David Artis[1,2,24–26]

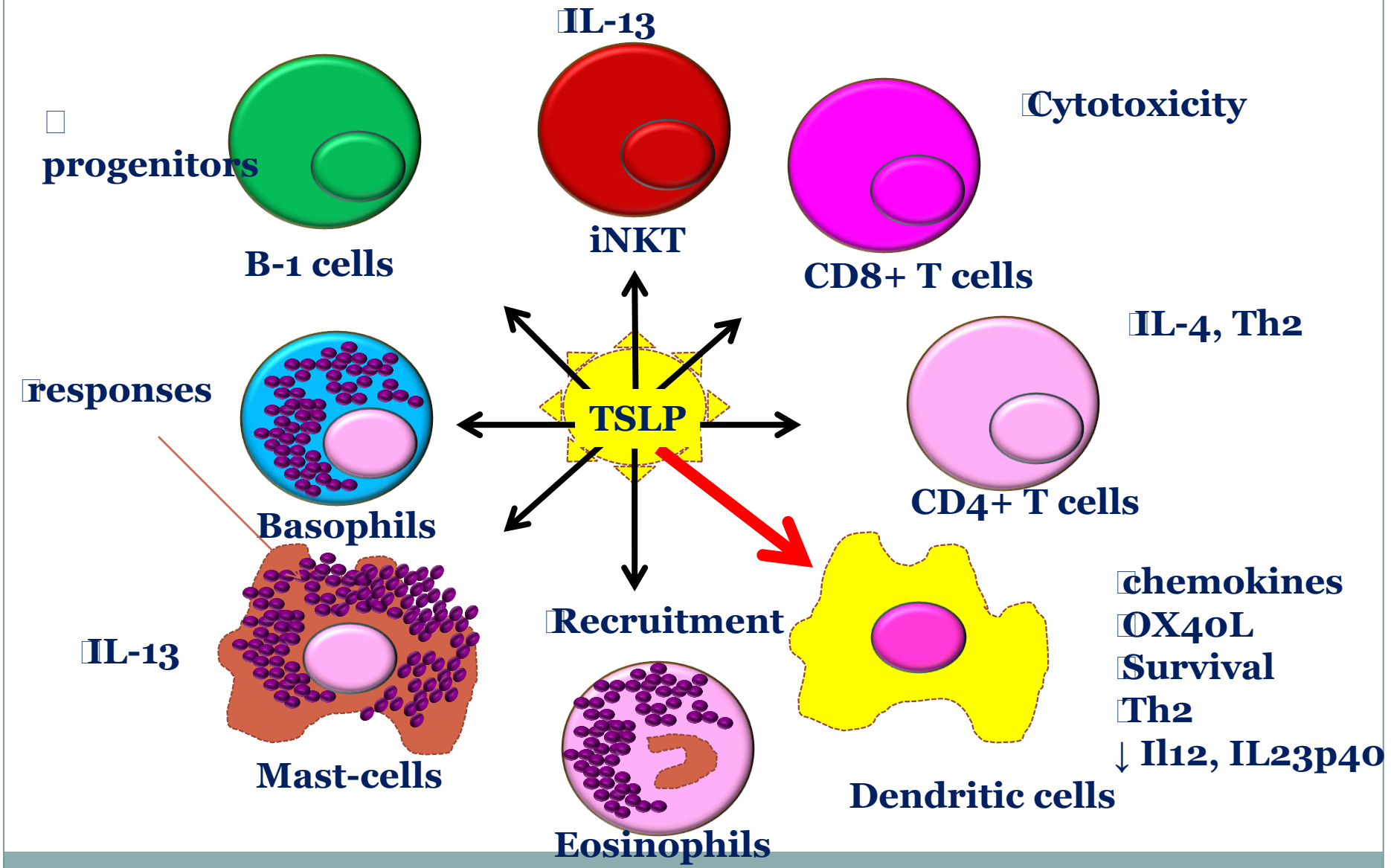
Basophil and TSLP dependent



Food impaction

Control IgG	Anti-TSLP	Anti-CD200R3
4/14 (29%)	0/9 (0%)	0/8 (0%)

TSLP-thymic stromal lymphopoietin



RS3806932 TSLP SNP is associated with multiple food allergy in EoE

381 biopsy confirmed pediatric EoE patients.

TSLP Risk Allele (homozygous/heterozygous)	Odds Ratio	Standard Error	P value	95% Confidence Interval
≥ 2 EoE food allergen triggers	4.616825	1.626283	<0.0001	2.314763-9.208321
Asthma	0.8805519	0.3394478	0.741	0.4136391-1.874513
AR	1.1322	0.496835	0.777	0.4790672-2.675775
AD	0.6805757	0.2524066	0.299	0.3289952-1.407873
IgE-FA	0.6867766	0.2398334	0.282	0.3463875-1.36166
Any Atopic Disease	1.653602	1.126703	0.460	0.4349646-6.286487
Male Gender	1.037716	0.2626875	0.884	0.6318373-1.704322

Fahey et at Clinical and Translational Gastroenterology 2018

CALPAIN 14 (CAPN14)

2 independent GWAS identified SNP at 2p23 as risk factor for EoE

CAPN14 is a functional proteases

CAPN14 is increased in the biopsies of patients with active EoE

Th2 Cytokines induce CAPN14 expression

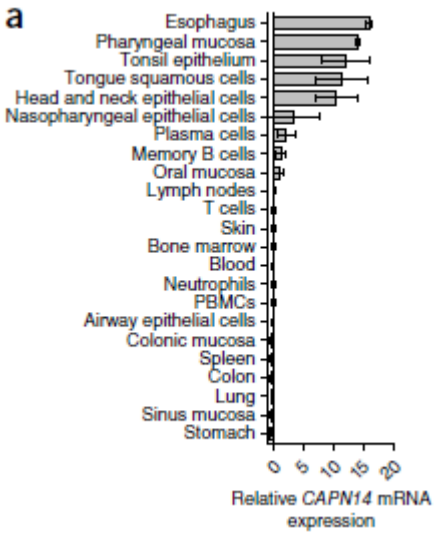
CAPN14 induces loss of *epithelial cell architecture and barrier function*

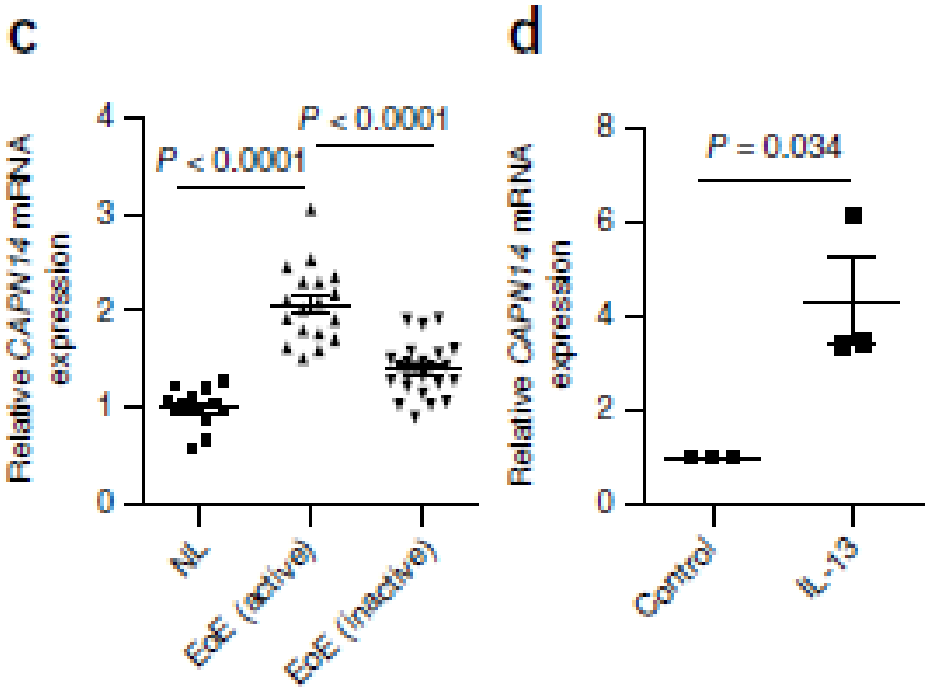
CAPN14 reduces Desmoglein 1 (DSG1) expression

Sleiman P et al 2014, Kattyan LC et al 2014, Davis BP et al 2016

Calpain 14 a proteasis and Th2 Inflammation Epithelial damage

CALPAIN 14

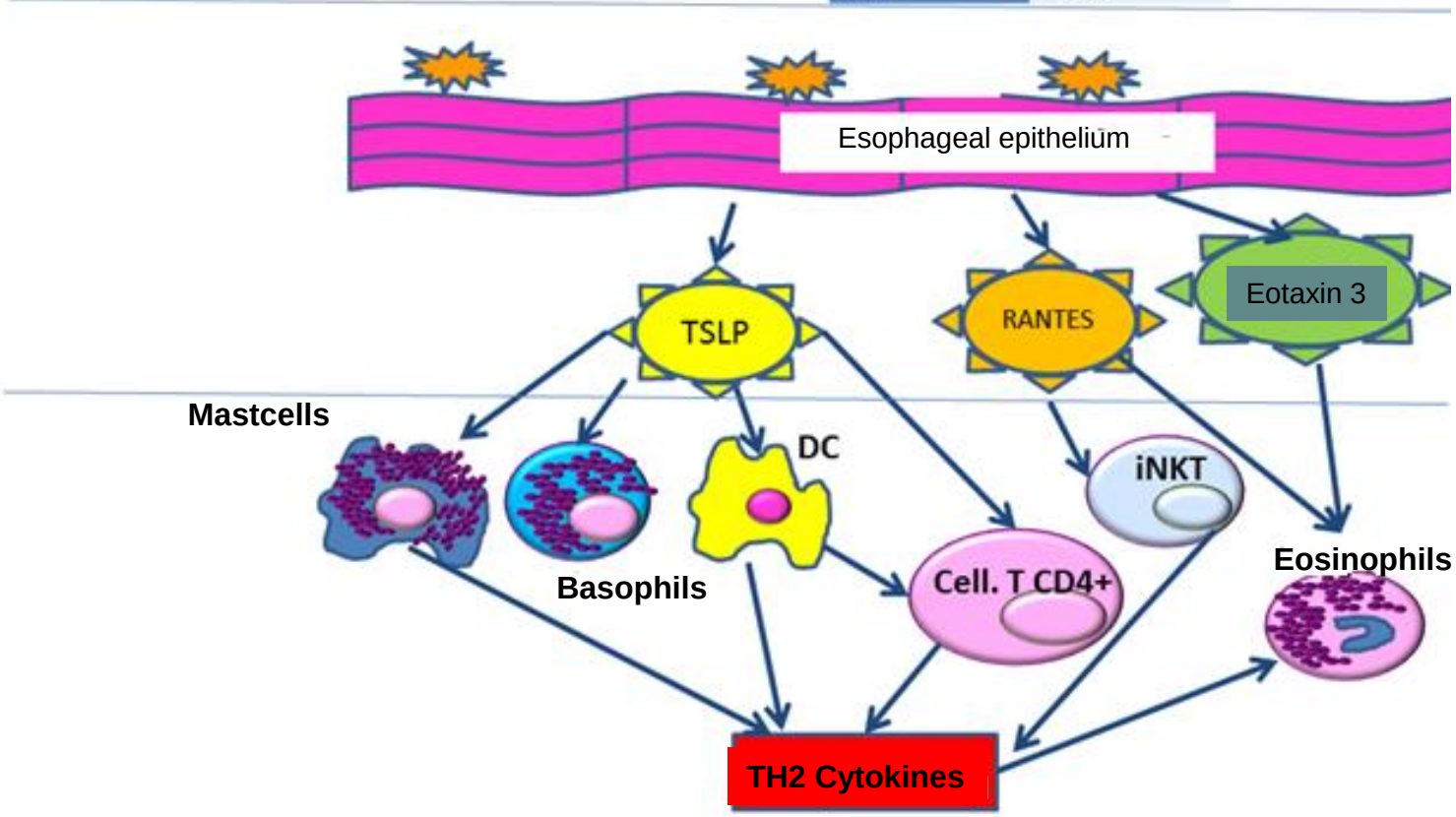




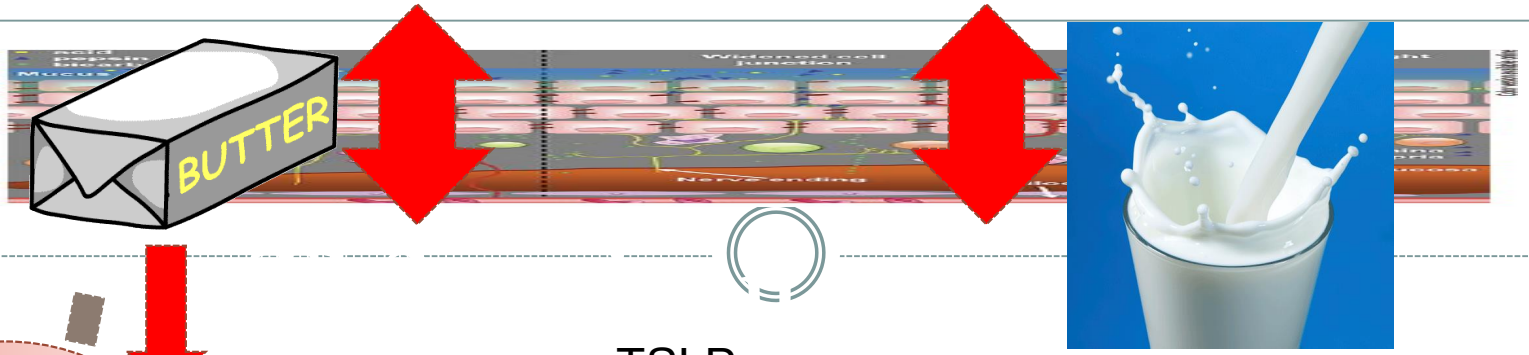
Kottyan 2014 Nature Genetics

EoE pathogenesis

TSLP SNP	5q.22.1
TSLP-R SNP	Xp22.3; Yp11.3
Eotaxin 3	7q11.23
CAPN14	2q.23
EMSY	11q13.5



Cianferoni A, 2015



TSLP

iNK T cell

Th2-Cytokine

Dendritic cells

Allergen - Protein

Th17

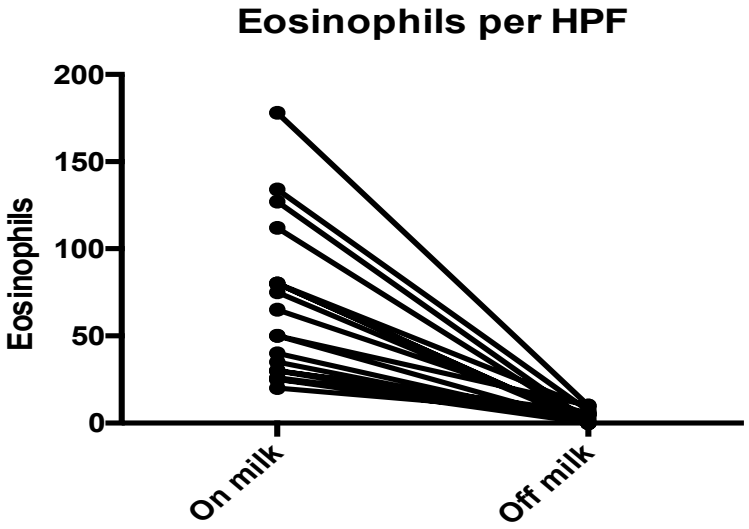
Th0

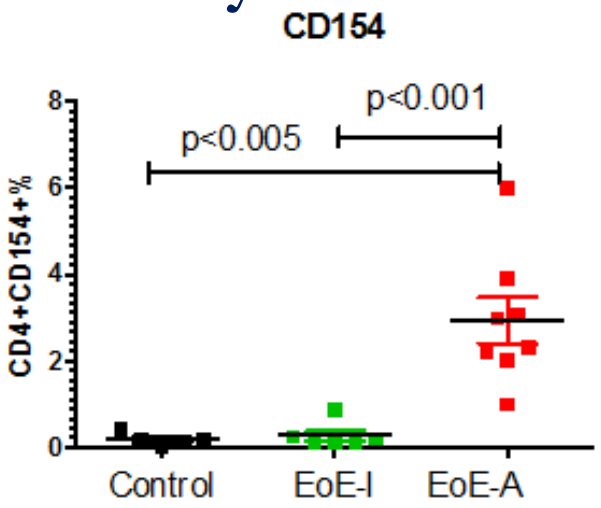
Th1

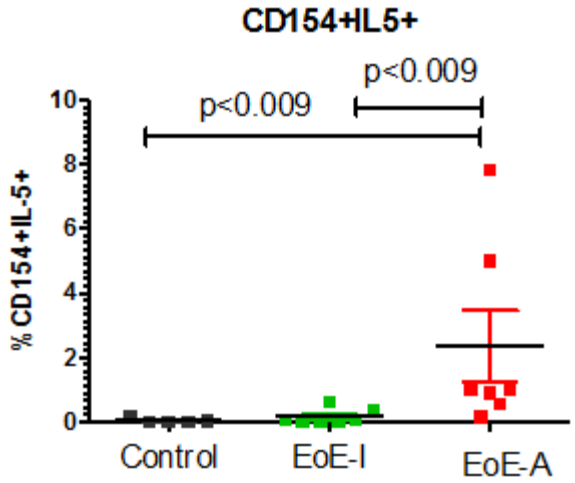
Th2

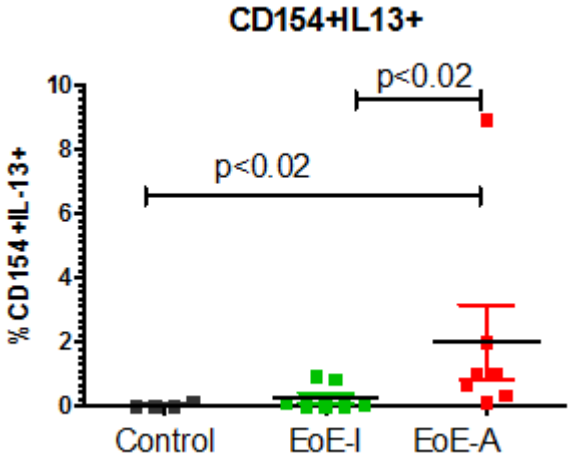
Treg

In 20 patients with milk induced EoE T cell were activated to produce Th2 cytokines



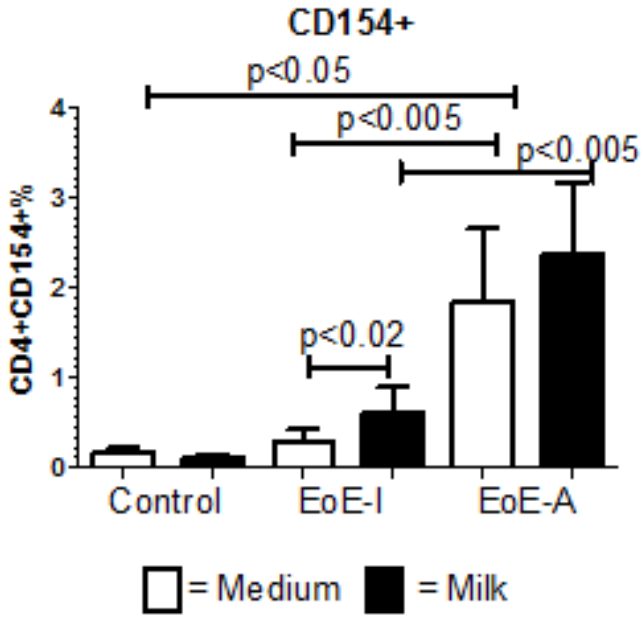


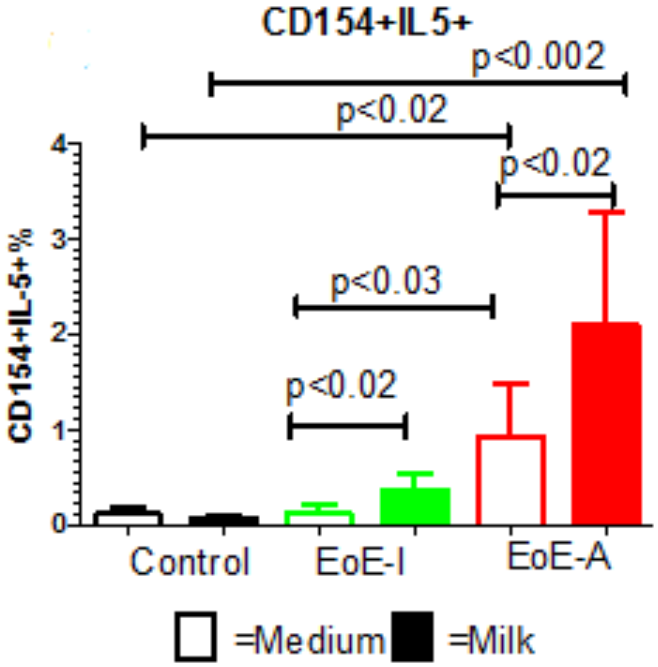




Cianferoni et al JACI submitted

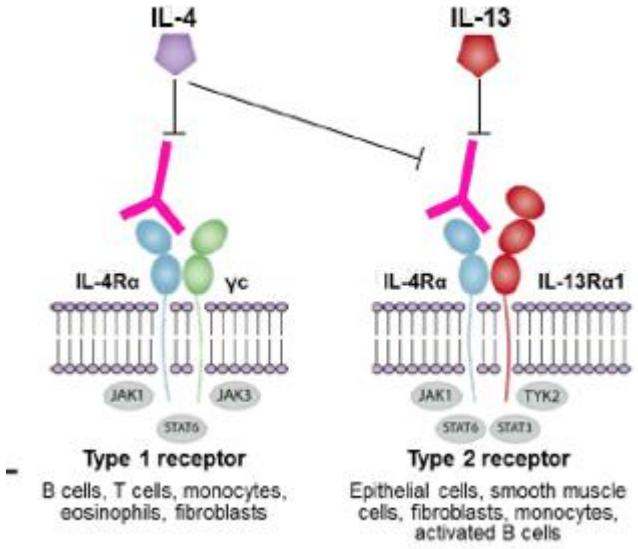
In patients with milk induced EoE T cell were activated to produce IL-5cytokines by milk antigens





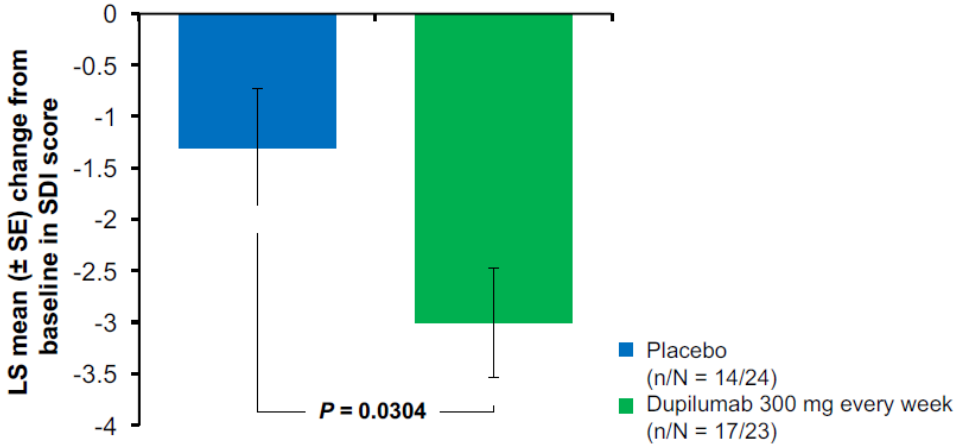
Cianferoni et al JACI submitted

Dupilumab (Anti-IL4R) in EoE



Primary Endpoint

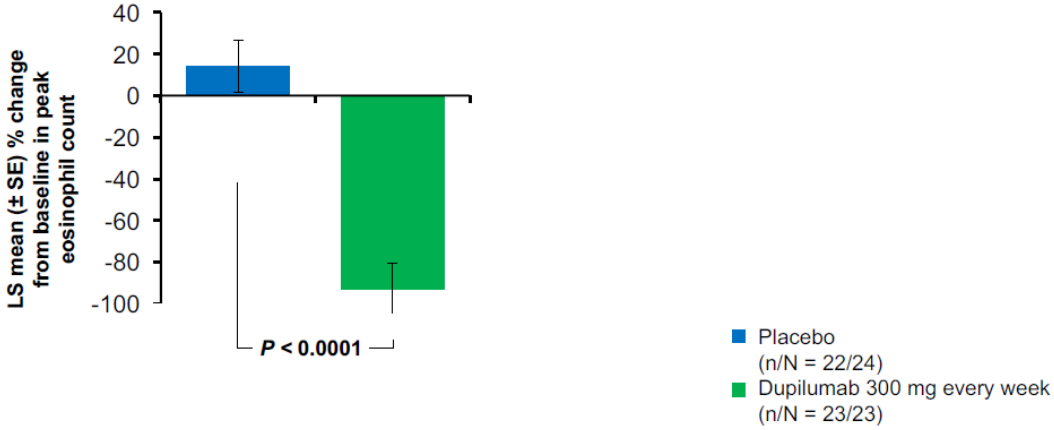
Dupilumab significantly reduced SDI PRO score at Week 10



.S, least-squares.

Secondary Endpoint

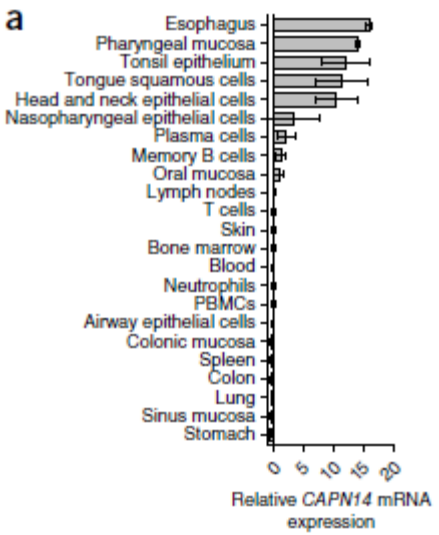
Dupilumab significantly reduced overall peak esophageal intraepithelial eosinophil count at Week 12

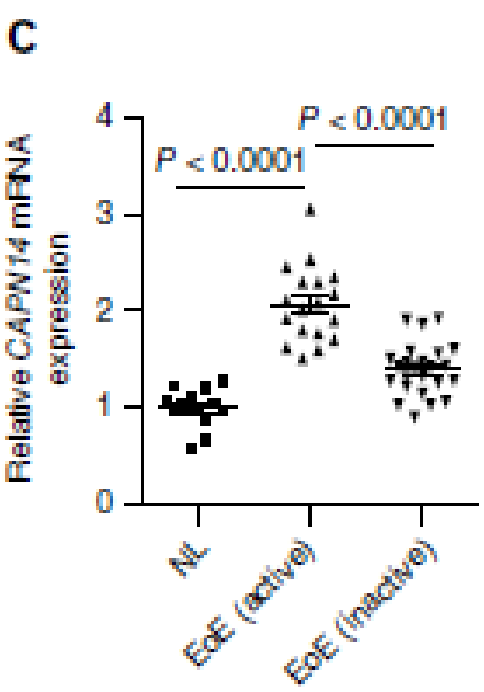


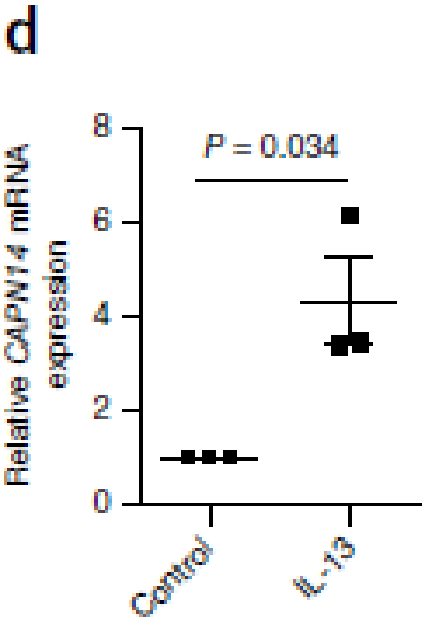
Th2 cytokines and epithelial dysfunction

Th2 Inflammation and Epithelial damage

CALPAIN 14

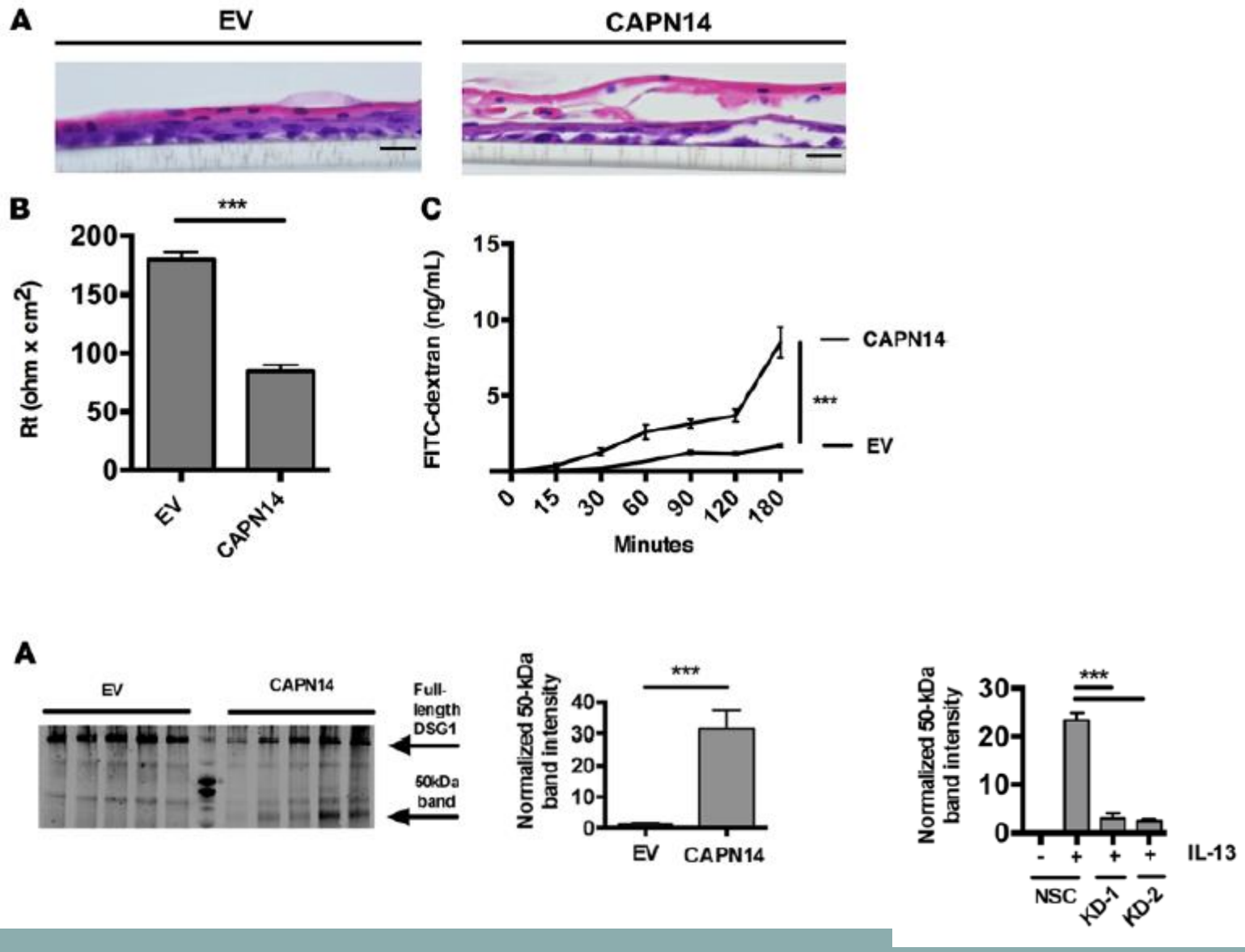




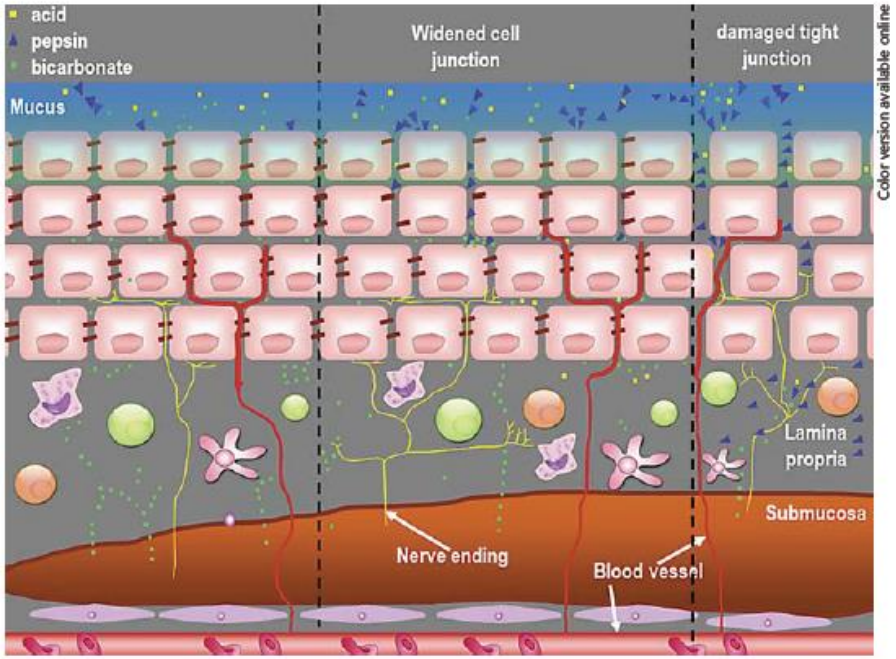


Kottyan 2014 Nature Genetics

Calpain 14 and Barrier dysfunction

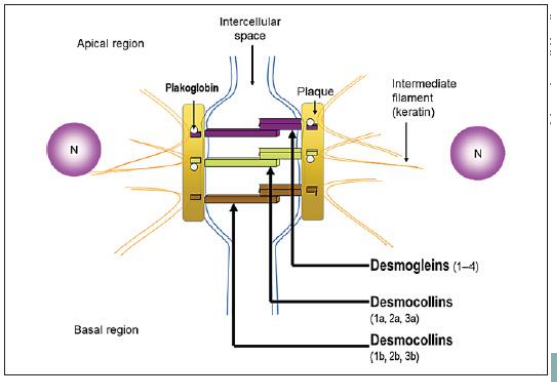


Esophageal Epithelial barrier



Multilayered stratified nonkeratinized quamous epithelium composed of the:

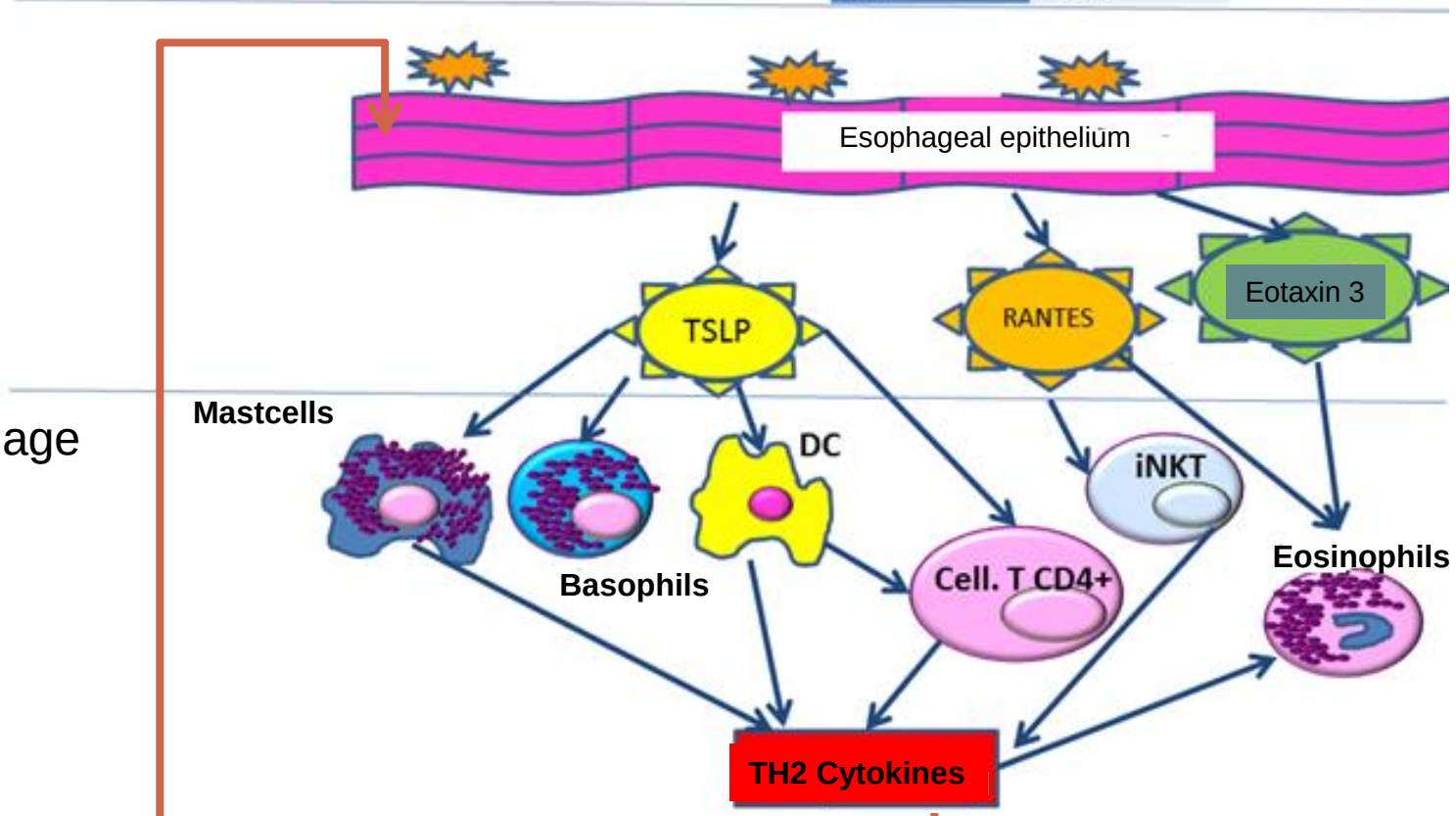
- stratum granulosum,
- stratum spinosum,
- stratum germinativum (i.e. basal layer; fig. 2).



- Tight junctions (occludins and claudins 1-4)
- .Adherent junctions (E-cadherin).
- Desmosomes (desmogelin and desmocollin)

EoE pathogenesis

TSLP SNP	5q.22.1
TSLP-R SNP	Xp22.3; Yp11.3
Eotaxin 3	7q11.23
CAPN14	2q.23
EMSY	11q13.5



Demage

Cianferoni A, 2015

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.

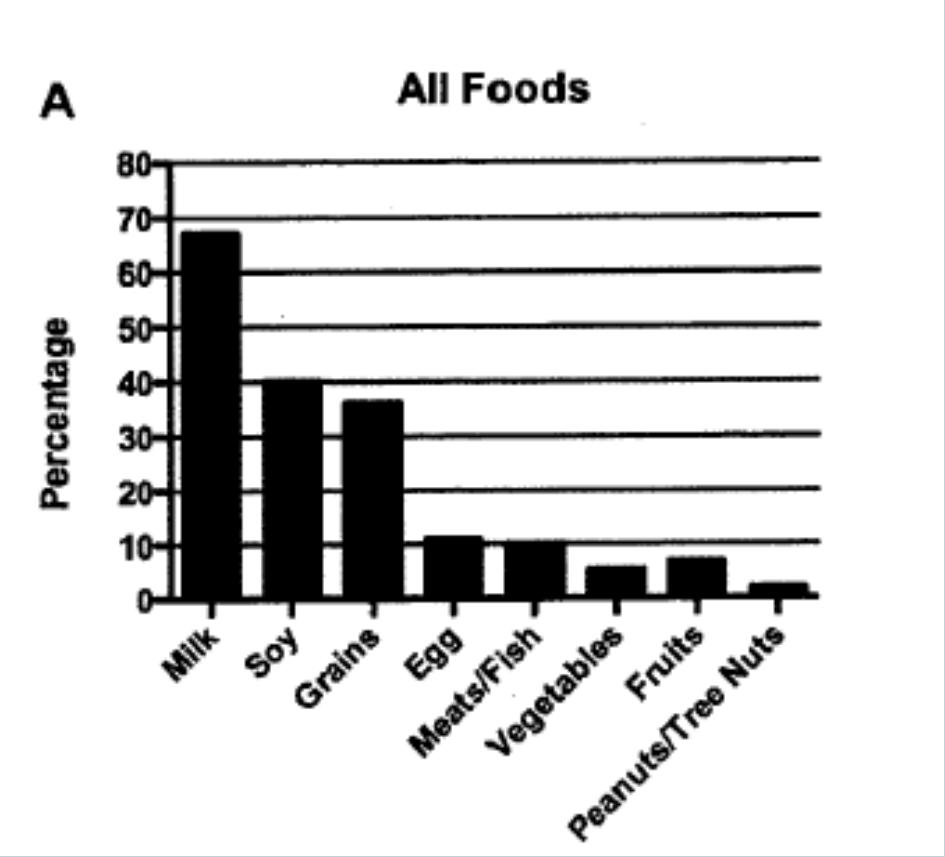
Atopy patch testing (APT) has also been evaluated as a possible means of identifying specific food sensitivities in patients with FPIES.

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 (JACI 2017)

FPIES: Foods

- 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)

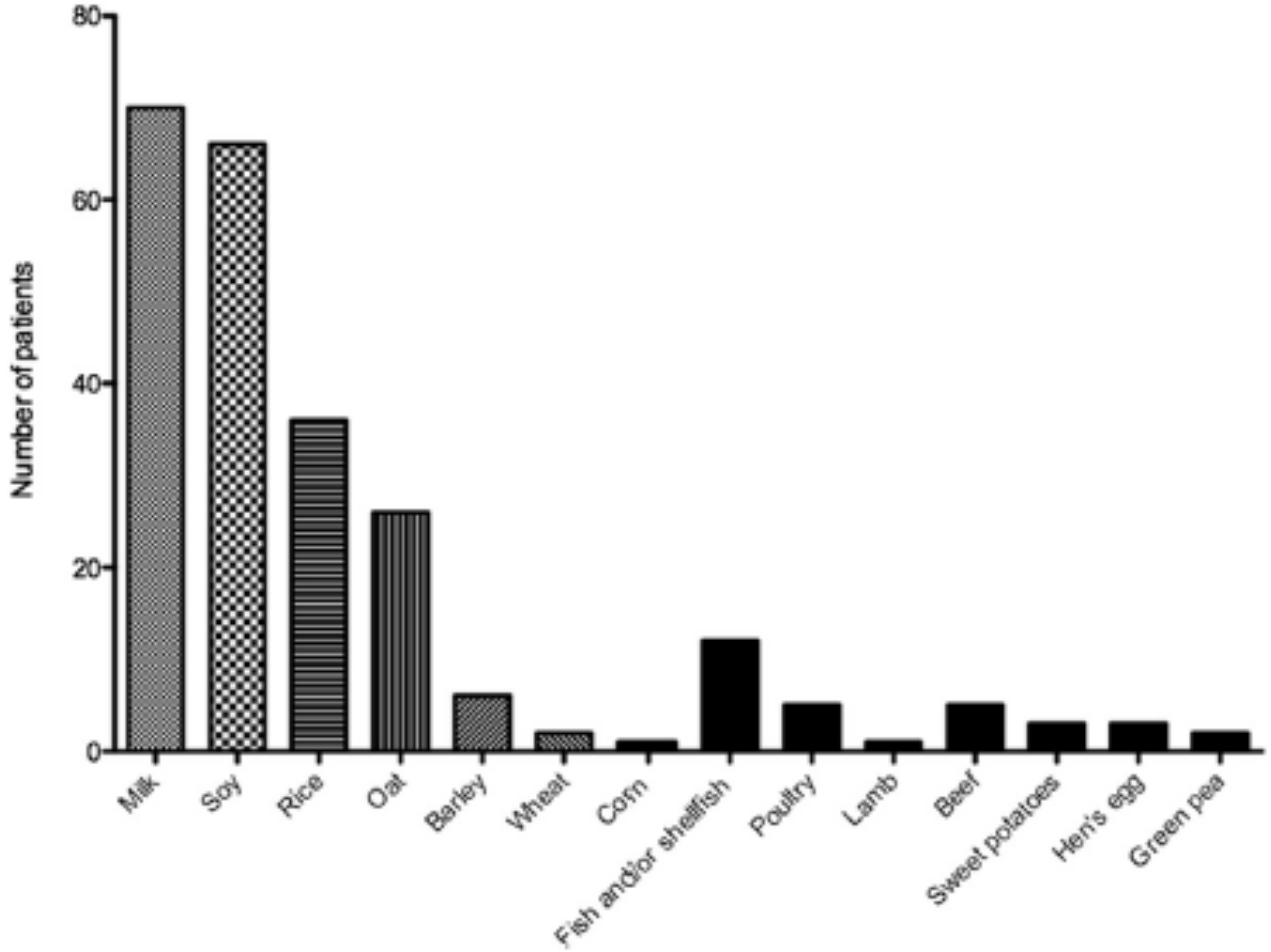
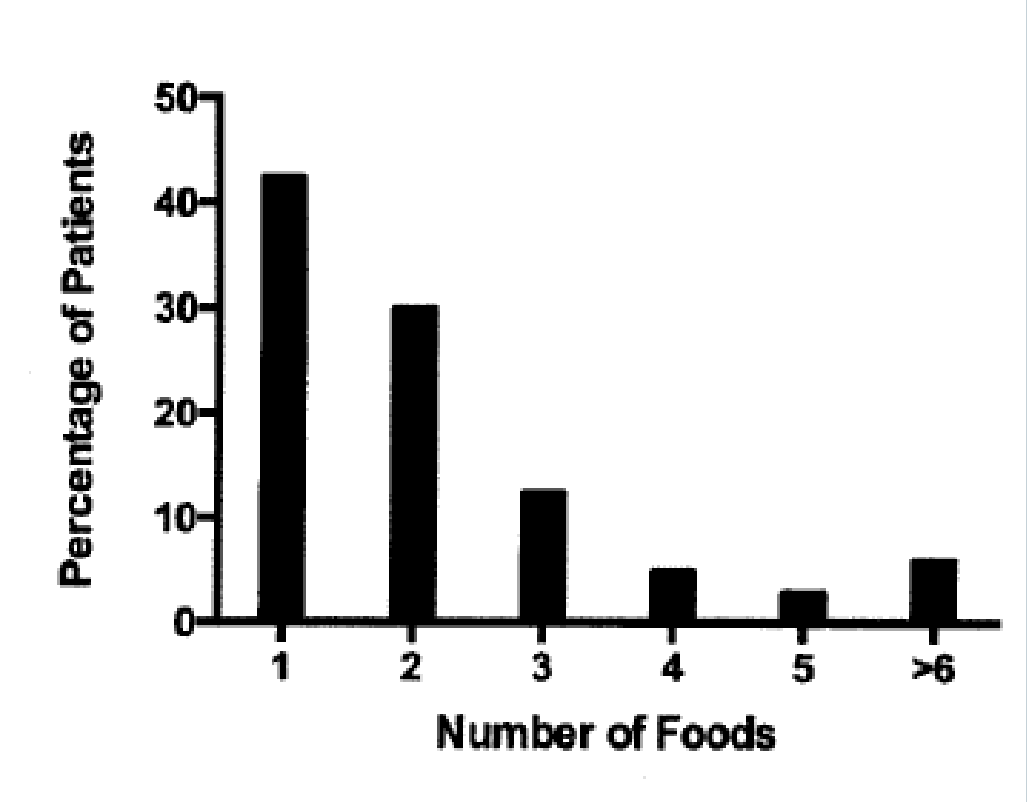


FIG 1. FPIES food triggers.

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

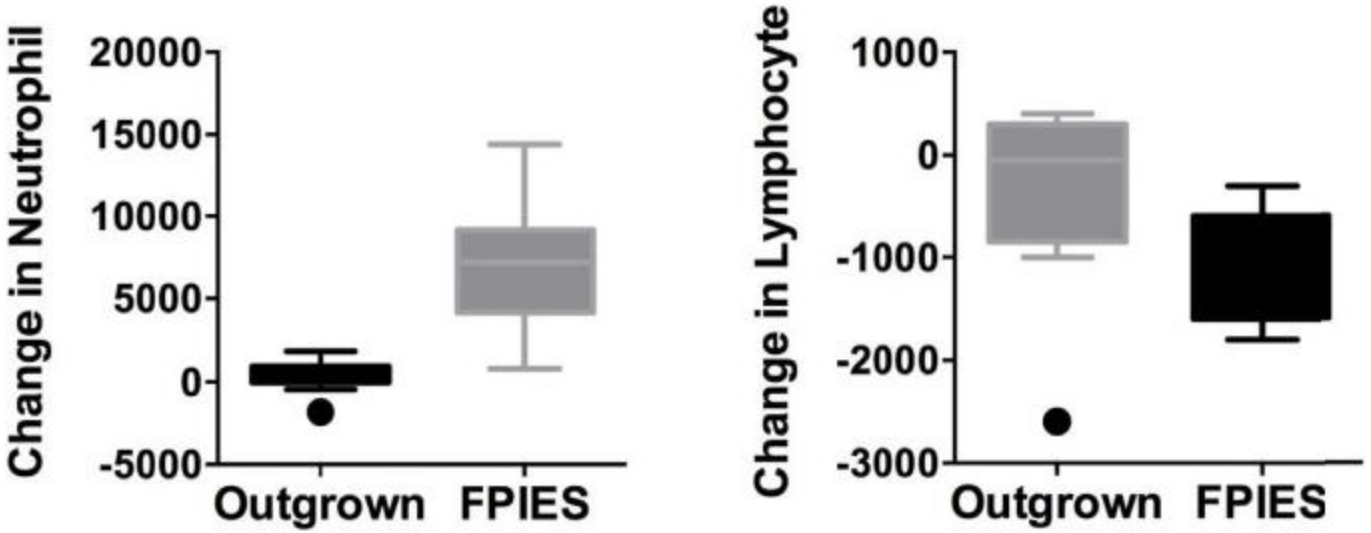
FPIES: Foods

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



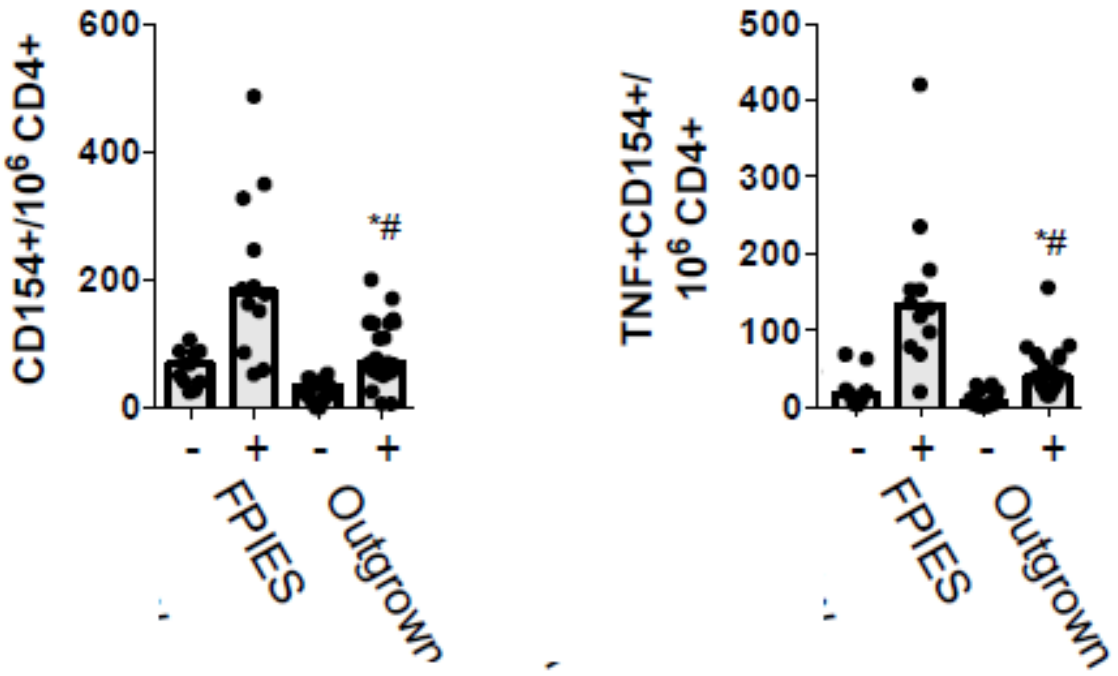
Ruffner et al JACI in practice 2014

T cell response to specific antigens in FPIES



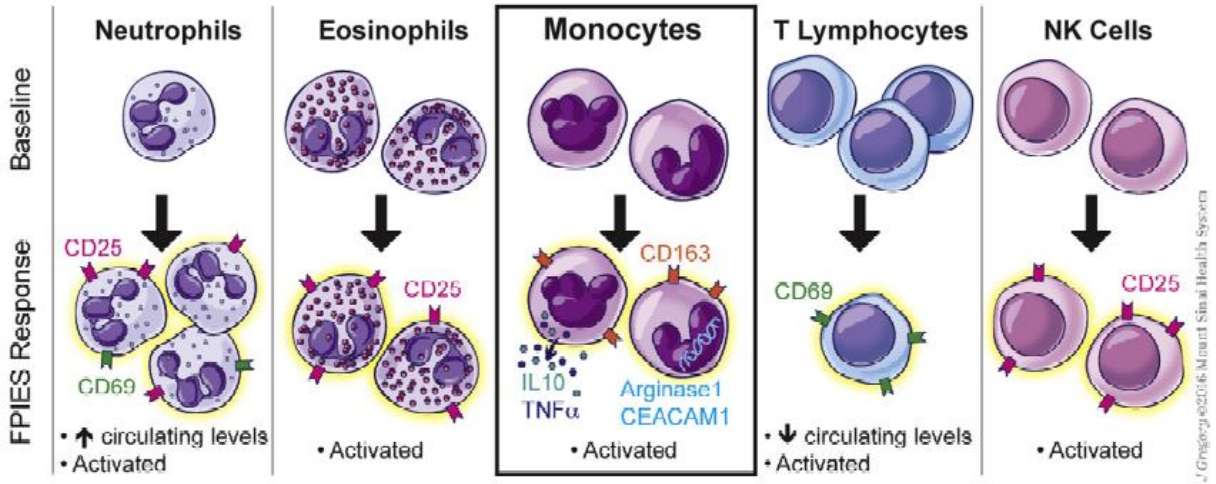
Goswami et al JACI 2017

T cell response to specific antigens in FPIES



Goswami et al JACI 2017

Innate immunity activation in FPIES



T cells

-milk, soy, or rice-responsive T cells, and TNF-α–producing CD1541 T cells, were significantly lower in those with outgrown FPIES compared with those with active FPIES. However, levels were within the normal range and were inconsistent with a role in the pathophysiology of FPIES.

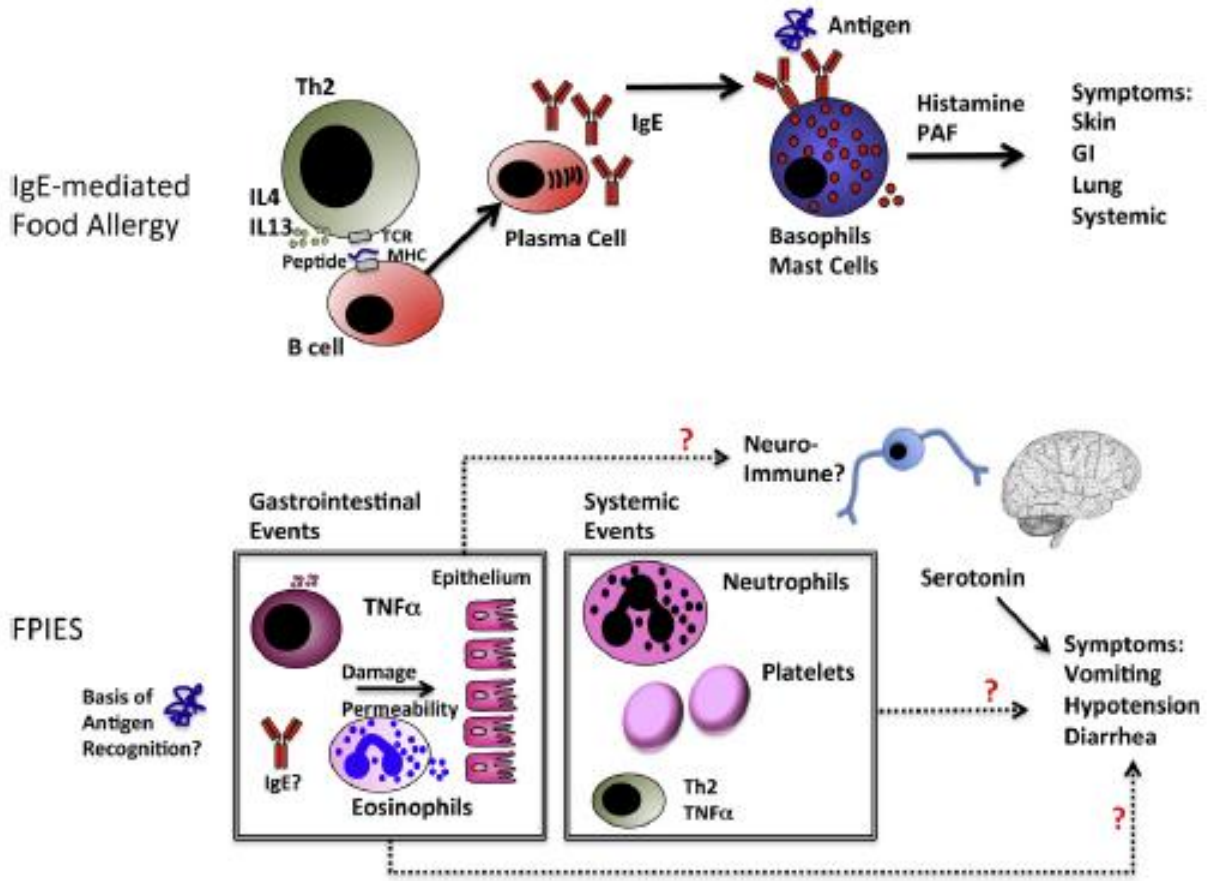
-pan–T-cell activation and redistribution from the circulation after a positive food challenge but not in those who had outgrown their FPIES

Innate immune system

-monocytes, neutrophils, natural killer cells, and eosinophils activated after food challenge only in children with active FPIES

Goswami et al JACI 2017

What are the mechanism that cause FPIES



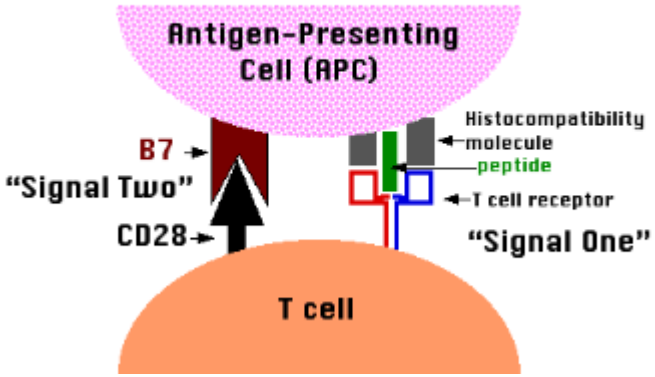
Berin C, JACI 2016.

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



Epithelium?
Antigen?
Genetics?
MHC?

IgE mediated

FPIES

EoE

B cell
IgE
Mastcells

Eos
Basophils
Mastcell
Epithelial damage

Mast cell
neutrophils

Modified from http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/A/AntigenPresentation.html

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/AAAAI
- FARE

IgE mediated Wheat allergy

Table 1 Allergens in wheat flour

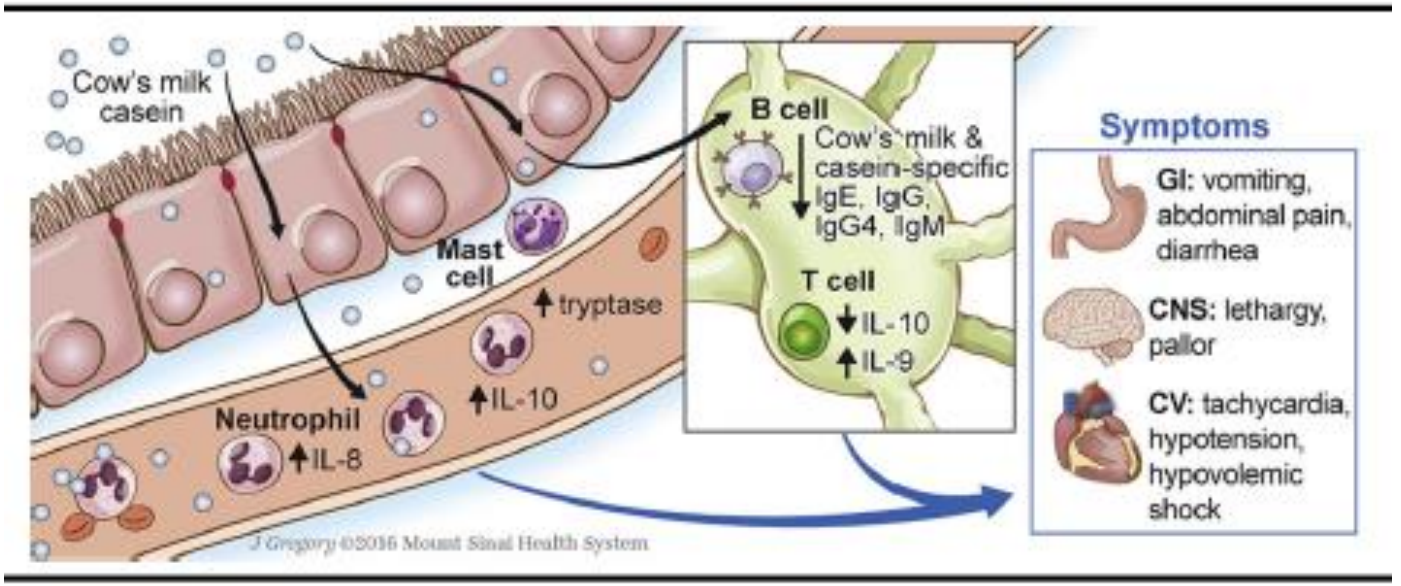
Allergen name	Allergen abbreviation	Molecular weight (kDa)	Reference
α-purothionin	Tri a 37	37	32–34
α-amylase/trypsin inhibitor	Tri a 28 and Tri a 29.01	12–16	5,29
Peroxidase	Tri a Bd36 kd	36	37,38
Thioredoxin	Tri a 25	25	39–41
Lipid protein transfer	Tri a 14	14	29,35,36,128
Serine proteinase inhibitor	Tri a 29	9.9	42,43
Thaumatin-like protein (TLP)		21–26	44
Gliadin	ω-5-gliadin	65	30,31,45
Thiol reductase	Tri a 27	27	128,129
1-cys-peroxiredoxin	Tri a 32		128,130
Serine protease like inhibitor	Tri a 39		128,130

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

Cianferoni A , J Asthma 2015, JACI, 2016

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

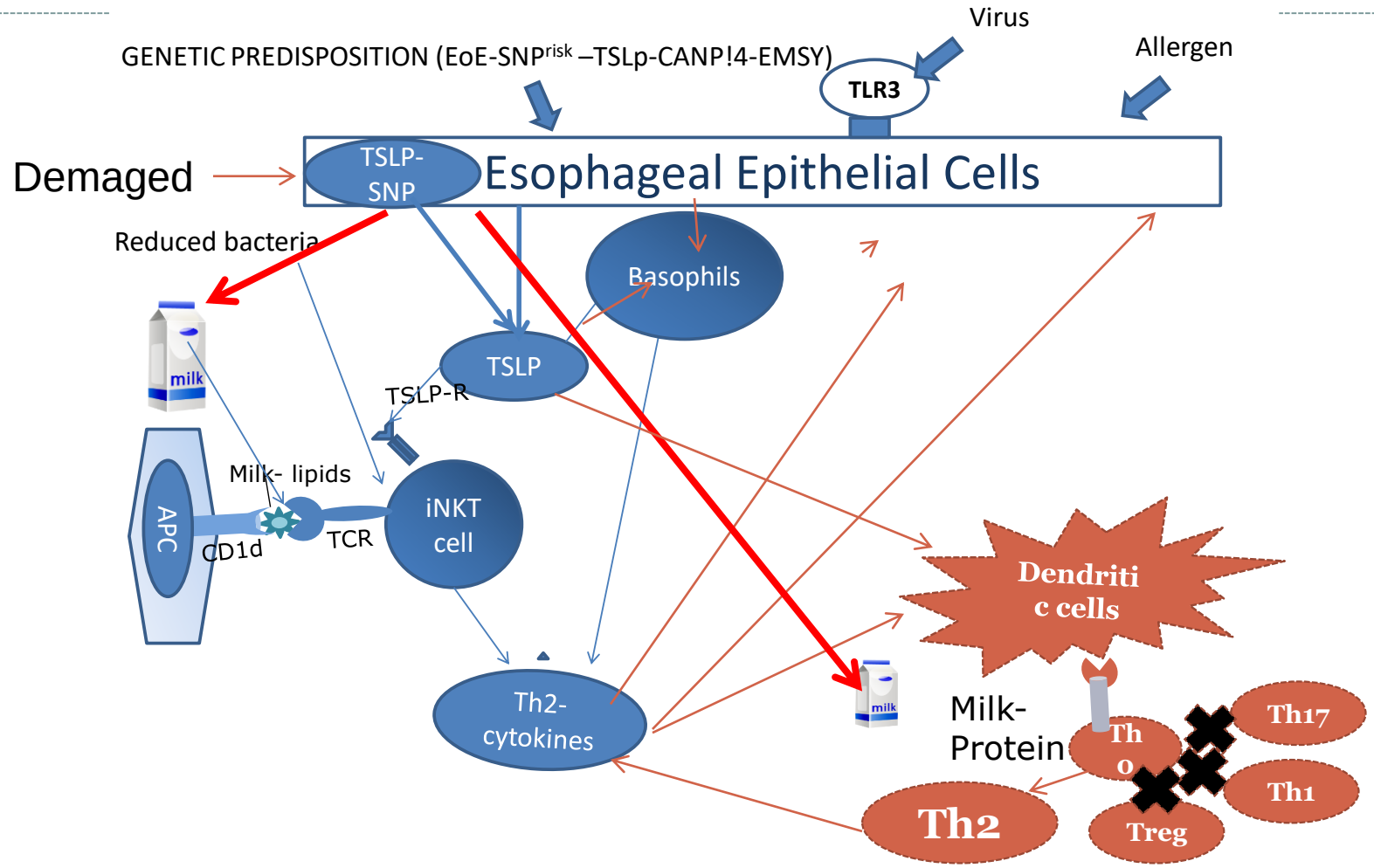
Humoral and cellular responses to casein in patients with food protein–induced enterocolitis to cow's milk

TABLE I. Summary of pathologic findings in patients with FPIES compared with control subjects

	Positive findings	Negative findings
Humoral responses	Lower levels of milk-specific IgG and IgG_4 in patients with CM-FPIES compared with those in patients tolerating CM (active vs resolved)	No difference in levels of specific and total IgM
	Trend toward lower ratios of casein-specific IgG_4/total IgG in patients with active CM-FPIES and patients with resolved CM-FPIES compared with those in patients with CM-tolerant FPIES	No difference in levels of specific IgD to casein, α-lactalbumin, and β-lactoglobulin
	Lower casein-specific IgM/total IgM ratio in patients with active CM-FPIES compared with that in patients tolerating CM	No difference in amounts of total κ Ig-fLC, λ Ig-fLC, CM-specific fLC, or λ Ig-fLC/κ Ig-fLC ratio
	Significantly lower milk-specific λ Ig-fLC/κ Ig-fLC ratio in patients with active CM-FPIES compared with that in patients tolerating CM	
T-cell and cytokine responses	Significantly lower secretion of IL-10 in patients with CM-FPIES compared with that in patients with IgE-CMA	Proliferating casein-specific $CD4^+$ T-cell frequency in patients with CM-FPIES similar to patients with IgE-CMA and control subjects
	Significantly higher IL-9–induced secretion in patients with CM-FPIES compared with that in patients with IgE-CMA	No difference found in T_H2 cytokine secretion, as well as IFN-γ and TNF-α secretion
Serum cytokines	At baseline: 1. Higher median concentration of IL-10 in patients with a negative OFC result compared with those with a positive OFC result 2. Higher median IP-10 concentration in patients with a positive OFC result	No change in the secretion of MCP-1, MIP-1α, and MIP-1β after a positive OFC result
	Significant increase in IL-10 and IL-8 secretion after a positive OFC result	
Tryptase level before and after an OFC	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	Serum tryptase levels not significantly different after a positive OFC result in patients with FPIES

MCP-1, Monocyte chemoattractant protein 1; *MIP*, macrophage inflammatory protein.

Model of Milk induced inflammation in EoE



Berin CM et al JACI 2008