Food allergies: from allergen to intestinal inflammation

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Food allergy: a significant problem

8.96% (7.64%) overall self-reported food allergy, 6.53% in children

Mc Gowan EC, Keet CA. JACI 2013

EAACI Food allergy & Anaphylaxis Guidelines Group:
Pooled life-time prevalence: self reported food allergy: 17.3%;
Point prevalence: specific IgE: 10.1%, SPT: 2.7%, pos. challenges: 0.9%

Nwaru BI et al. Allergy 2013

Germany DEGS1 study: Sensitization evidenced by specific IgE: >7000 blood samples 18-79yrs: overall 25.5% pos. results with 50 allergens tested

Haftenberger M et al. Bundesgesundheitsbl 2013
Hospitalisation due to food allergy

Average number of hospital discharges/year among children (under 18 years) with diagnosis „food allergy“ in the United States 1998-2006

http://www.cdc.gov/nchs/data/databriefs/db10_fig4.gif
Food allergy hospital admission

Overall food allergy admission rates: from 5 to 26/million
Children: 7-fold increase from 16 to 107/million
No data from ER included
Increase of admission rates: 500% since 1990

Economic burden by food allergy

Overall food allergy costs: $24.8 billion/year
$4,184 per child and year

Total costs included
$4.3 bill. direct medical costs
$20.5 bill. costs to families

Gupta R et al. JAMA Pediatr 2013

Health care cost for Europeans (EuroPrevall studies in 9 countries):

for food-allergic adults  $2,016 mean cost/year
for controls             $1,089 mean cost/year

No differences for adults and children in each country significantly related to disease severity

Fox M et al. Eur J Public Health 2013
EU legislation on food allergens

Labelling rules for 14 food allergens:

• Eggs
• Milk
• Fish
• Crustaceans
• Mollusks
• Peanuts
• 10 different tree nuts
• Sesame seeds
• Cereals containing gluten
• Soybeans
• Celery and celeriac
• Mustard
• Lupine
• Sulphites SO₂ (>10 mg/kg)
More than 170 food ingredients cause IgE-mediated reactions

Reviewed in Burks AW et al. JACI 2012
Characteristics of food allergens

- glycoproteins
- low dose → activation of IL4-producing CD4-T-cells
- low molecular weight (10-70 kDa) → diffusion through epithelia facilitated
- solubility
- stability to heat, acid, proteases
- some are enzyme inhibitors
- abundance in food

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Known allergen or gen source allergen?

Sequence homology, databank search

Allergological testing

Stability to digestion/processing

(Animal model)

http://usinfo.state.gov/products/pubs/biotech/
Simulated gastric fluid experiments

Food proteins + Gastric enzymes pH2
Digestion-labile food allergens

Biological activity of digested codfish

HR test: passive sens. basophils

10.900-times reduction of IgE binding capacity

Untersmayr E et al. JACI 2005
Pepsin and gastric acid producing cells

- Foveolar cells: mucus production
- Parietal cells: hydrochloric acid, intrinsic factor
- Chief cells: Pepsinogen
- G-cells: gastrin secretion

Physiological gastric digestion

Transformation of pepsinogen into the mature enzyme pepsin

Protein binding in substrate cleft and protein cleavage

Untersmayr E, Jensen-Jarolim E. COAI 2006
Untersmayr E, Jensen-Jarolim E. JACI 2008
Physiological protein digestion

Releasing of acidic chyme into the duodenum

⇒

Activation of duodenal (and jejunal) S-cells → secretin release

⇒

Stimulation pancreas proteases trypsin, chymotrypsin, carboxypeptidases release

Reviewed in Pali-Schöll I, Jensen-Jarolim E. Allergy 2011

Physiological protein digestion

Low gastric pH is decisive for activation and release of gastric and pancreas proteases
Hypoacidity and gastric digestion

Untersmayr E et al. JACI 2005, Untersmayr E et al. FASEB J 2005
Gastric hypoacidity in health and disease

Physiological: Newborns

Atrophic gastritis

Pharmacological:
• Antacids & Sucralfate
• H2 receptor blockers
• Proton pump inhibitors

http://www.kur-apotheke-wolter.de/info-magen.html
Acid suppression medication – mode of action

H2-receptor blockers

Proton pump inhibitors

Antacids: slight bases neutralizing gastric acid

Sucralfate: Aluminum compound → strong neg. charge upon Al³⁺ release

Murine experiments

digestion-labile food allergens +
Sucralfate
H2 receptor blockers
Proton pump inhibitors
Rennie®
Base powder

Untersmayr E et al. JACI 2003
Schöll I et al. Am J Clin Nutr 2005
Schöll I et al. FASEB J 2007
Untersmayr E et al. Mech Ageing Dev. 2008
Brunner R et al. Allergy. 2009
Riemer AB et al. Clin Exp Allergy 2010
Pali-Schöll I et al. Clin Exp Allergy. 2010
Untersmayr et al. PLoS One 2010
Krishnamurthy D et al. Ms submitted
Diesner SC et al. Ms submitted
Microscopic analysis of gastrointestinal mucosa

Eosinophils

Intestinal architecture
Codfish + sucralfate

Mast cells

Untersmayr E et al. JACI 2003
Untersmayr et al. IAAI 2004
Dieser S et al. Imm Lett 2012

Pali-Schöll I et al. Exp Toxicol Pathol. 2008
Human studies

Observational study: patients with dyspeptic disorders treated with anti-ulcer medication

Allergologic evaluation of elderly

Untersmayr E et al. FASEB J 2005
Schöll et al. Am J Clin Nutr 2005
Bakos N et al. Imm Letters 2006
Changed reactivity profile in allergic patients

Digestion significantly reduces skin reactivity

Non-digestion reduces threshold levels

Untersmayr E et al. JACI 2007
The role of gastric digestion in food allergy

Population based health register study: Association between maternal anti-ulcer intake and childhood asthma

Dehlink E et al. Clin Exp Allergy 2009

Questionnaire study: correlation between increasing prevalence of childhood food allergy anti-ulcer medication

Karen DeMuth et al. Allergy and Asthma Proceedings 2013

Insurance data base analysis (14.172 children between 0-18 ys): acid suppression associated with a higher food allergy risk

Trikha A et al. Pediatric Allergy Immunol 2013

Untersmayr E, Jensen-Jarolim E. JACI 2008
Food allergy model in BALB/c mice

Oral immunizations with digestion-labile allergens under concomitant gastric acid suppression

- 1st immunization
- 2nd immunization
- 3rd immunization
- Read-out experiments

Antiacids

Intragastric pH

normal

Antiacid

*
Food allergy against OVA proteins

Allergen factors: environmental protein nitration

Chemical modification of airborne proteins by NO$_2$ and O$_3$

*Shiraiwa M et al. Nature Chem 2011*

Nitrated Bet v1 in urban dust samples

*Franze T et al. Environ Sci Technol 2005*

Conformational change in nitrated proteins

*Souza JM et al. Arch Biochem Biophys 2000*

Enhanced allergenic potential of nitrated Bet v1 for inducing IgE-mediated type I allergy

*Gruijthuijsen YK et al. IAAI 2006*
Endogenous protein nitration

- Endogenous protein nitration during inflammation

  Extent of protein nitration was considered as a maker of oxidative stress in inflammatory responses

  Yeo WS et al. BMB reports 2008

  Helicobacter pylori gastritis or inflammatory bowel disease


- Gastrointestinal environment

  Nitrous acid is formed only at low pH → acidic gastric milieu required for nitration


  Nitrated proteins cleaved slower by chymotrypsin than untreated proteins


http://bodybymitch.com/page/2/
Immunization regimen

Day 0  14  28  42  56  70  84  98  112

- PPI + oral imm.
- sham-nitrated OVA
- nitrated OVA

untreated OVA
untreated OVA
untreated OVA

PIS  MIS 1  MIS 2  MIS 3  MIS 4  MIS 5  MIS 6

Day 0  14  28  42  56  70  84  98  112

- b. imm.
- sham-nitrated OVA
- nitrated OVA

untreated OVA
untreated OVA
untreated OVA

PIS  MIS 1  MIS 2  MIS 3  MIS 4  MIS 5  MIS 6
Induction of OVA specific IgE
Allergenicity of nitrated OVA

untreated OVA
nitrated OVA
nitrated Bet v1

untreated OVA
sham OVA
nitrated OVA
naive

β-hexosaminidase (%)

anti-acid oral ip. anti-acid oral ip. anti-acid oral ip.
SGF assays with OVA preparations

- **Untreated OVA**
  - pH1
  - pH3
  - pH5

- **Sham-nitrated OVA**
  - pH1
  - pH3
  - pH5

- **Nitrated OVA**
  - pH1
  - pH3
  - pH5
Characteristics of nitrated OVA

Pepsin interaction partners: phenylalanine, tyrosine and leucine

MGSIGAASMEFCFDVFKELKVVHANENIFIYCPIAIMSALAMVYLGAKD
STRTQINKVVRFDKLPGFGDSIEAQCGTSVNHVHSLRDLILNQIYKPD
YSFSLASRLYAEERPILPEYLCVYELYRGGLEPINFQTAADQARE
LSNWVESQTNGIIRNLQPSSVDSQTAMVLVNAIFKGLWEEKTFKDE
YTLQAMPFRVTSEQESKPVQMMYQIGLFRVASMASEKMKILELPFASGT
MSMLVLLPDEVSGLEQLEGSIINFEKLTETWTSNVMEEERKIKY
YLPRMKMEEKYNLTSVLMAMGITDVFSOUTHNLGASSAESLKLISQAVHAAHAEEI
EAGREVVGSAAEAGVDAASSVSEEFRADHPFLFCIKHIATNAVLFFGRCV

10 tyrosine residues: 21% nitration grade

Y$_{107}$ nitration degree: 1
Y$_{112}$ nitration degree: 0.11
Y$_{282}$ nitration degree: 0.14
OVA epitopes and IgE recognition

MGSIGAASMEFCFDVFKEKLVVHANENIFYCPIAIMSA/LAMVYLGAKDSTRTQINKVVRFDKL/P/GFSDIEAQCGTSNVHSSLRDILNQIYKPNDSYSFLASRLV107: YAEERYPILPEYLQCVKELYRGGLEPINFQTAADQARELINSWVESQTNGIIRNLQPSSVDSQTAGMLVNAVFKGLWETKDFEMSSMLVLLPDEVSGLEQLESIINFEKLTETWTSSVMEERKIKVYPRMK MEEKYNLTSVLMAMGTDFVSSSANLIGISSAELKISQAVHAAHAEINEAGREVVGSAEAGVDAASVSEEFRADHPFLFCIKHIATNAVLFGRCV

B-cell epitope
T-cell epitope

Nitrated Y\textsubscript{107}:
- B-cell and T-cell epitope
- IgE epitope recognized exclusively after oral immunization

Mine Y et al. BBRC 2009
Mine Y et al. BBA 2007
Protein nitration in food allergy

- Differences in administration routes in experimental models of food allergy
- Enhanced triggering capacity of nitrated OVA proteins in allergic animals
- Reduced *de novo* sensitization capacity of nitrated OVA via the oral route due to enhanced digestibility underlining the importance of gastric digestion in food allergy and/or changes in antibody epitopes

*Untersmayr E et al. PLoS One 2010*
Structure impacts on food uptake

~2% intact proteins reach intestinal lymph nodes and portal circulation

Warshaw AL et al. Gastroenterology 1974

Non-sensitized individuals: protein uptake via M-cells and enterocytes

Food protein uptake in allergy

Reviewed in Reitsma M et al. Mol Nutr Food Res 2014

sensitized individuals: paracellular route involvement of Mast cells
transcellular route via enterocytes
Intestinal microbiota and allergy

Germ-free mice: immunodeficient, without colonization no oral tolerance development, decreased intestinal surface, decreased cell renewal, thinner mucus layer

Differences in gut microbiota between atopic and healthy persons

Infants with food allergy reveal imbalance between beneficial and potentially harmful bacteria

Bacterial strain specific Fox p3+ Treg induction in vivo protects against airway inflammation and oral IgE induction in mouse allergy models
Lyons A et al. Clin Exp Allergy 2010
Contributors to food allergic gastrointestinal inflammation

- Route of allergen entry
- Microbiota composition
- Inflammatory mediators and signaling molecules

http://www.coreonehealth.com/gastrointestinal
Intestinal food allergic response

Lamichhane A et al. J Gastroenterol Hepatol 2013
Sphingolipids

- Large family of lipids
- Ubiquititary expressed
- Constituent of cell membranes and lipid rafts on the outer leaflet of the plasma membrane

General chemical structure of Sphingolipids

Pierce SK. Nature Reviews Immunology 2002
Sphingosine-1-Phosphate

- S1P is produced in various cell types and impacts on apoptosis, cell migration, cell growth, immune cell activation
- Exogenous introduction of SM via food → de novo synthesis
- Endogenous production

Endogenous S1P production

Mainly in endothelial cells, erythrocytes, platelets, mast cells

Sphingosine

Sphingosine kinases (SphK1 and SphK2)

S1P

Role of S1P in allergic inflammation

Ossetitzian CA et al. Pharmacol Ther 2007
Sphingolipids and food allergy

Intestinal tract relatively rich in sphingolipids (~30% of total lipids in apical membrane of villi, being higher than basolateral)

\[ \text{Kurashima Y et al. J Immunol 2007} \]

\[ \text{Danielsen EM et al. Mol Membr Biol 2007} \]

S1P-dependent recruiting of systemically primed CD4+ T-cells from spleen to intestine → antigen-specific intestinal allergic hypersensitivity in mice

S1P-mediated migration of mast cells with specific IgE towards antigen

\[ \text{Kurashima Y et al. J Immunol 2007} \]

\[ \text{Kunisawa J et al. Nutrients 2012} \]
S1P increases the transepithelial allergen uptake \textit{in vitro}

Impact of SphK1 and SphK2 deletion on food allergy

Food allergic group

Negative control group

Positive control

OVA ig. + ANT

Day 0 3 14 17 28 30 42 44 59

OVA ig.

Day 0 3 14 17 28 30 42 44 59

OVA ip.

Day 0 3 14 17 28 30 42 44 59

PPI + oral Imm.

PPI + oral Imm.

PPI + oral Imm.

PPI + oral Imm.

Oral Challenge

Aerosol Challenge

Impact of SphK1 and SphK2 deletion on food allergy
SphK deletion inhibits oral OVA sensitization
Anaphylactic response is aberrant in SphKs deleted animals.
SphK2 deletion enhances systemic mast cells

Flow cytometry analysis of spleen cell subpopulations

Mast cells (c-Kit+ FcεRI+)

OVA ig.+ANT

OVA ig.

OVA ip.

WT SphK1 SphK2

WT SphK1 SphK2

WT SphK1 SphK2
SphK deletion decreases mast cell accumulation in gastric mucosa
Proposed mechanism

→ S1P homeostasis influences food allergy development and effector cell function

S1P homeostasis in the gut

Shingolipid homeostasis in the gut


Izawa K et al. JACI 2014
Diverse contributors to food allergy

**Individual factors**
- Digestion
- Microbiota
- Mediators
- Genetics, Epigenetics, ..

**Allergen factors**
- Structure
- Stability
- Modifications

Complex network of contributing factors
Thank you!

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