Toxicology and Food Allergy: Case Study of tBHQ

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Outline

- Trends in food allergy
- Immune response during allergy
- Evaluation of allergenicity of novel proteins
- Animal models of food allergy
- Toxicity testing in food allergy case study: tBHQ
Trends in Food Allergy

Four out of every 100 children have a food allergy.

Figure 1. Percentage of children under age 18 years who had a reported food or digestive allergy in the past 12 months, by age, sex, and race and ethnicity group: United States, 2007

- Total: 3.9%
- Less than 5 years: 14.7%
- 5-17 years: 3.7%
- Male: 3.8%
- Female: 4.1%
- Non-Hispanic white: 4.1%
- Non-Hispanic black: 4.0%
- Hispanic: 3.1%

Notes:
1. Significantly different from children aged 5-17 years.
2. Significantly different from non-Hispanic white and non-Hispanic black children.
SOURCE: CDC/NCHS, National Health Interview Survey.
Trends in Food Allergy

Figure 2. Percentage of children under age 18 years who had a reported food or digestive allergy in the past 12 months, by age group: United States, 1997–2007.

1Statistically significant trend.
SOURCE: CDC/NCHS, National Health Interview Survey.
Trends in Food Allergy

Figure 3. Percentage of children under age 18 years with asthma or other reported allergic conditions in the previous 12 months, by reported food allergy status: United States, 2007

SOURCE: CDC/NCHS, National Health Interview Survey.
Trends in Food Allergy

Figure 4. Average number of hospital discharges per year among children under age 18 years with any diagnosis related to food allergy: United States, 1998–2006

<table>
<thead>
<tr>
<th>Years</th>
<th>Average number of discharges per year</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998–2000</td>
<td>2,615</td>
</tr>
<tr>
<td>2001–2003</td>
<td>4,135</td>
</tr>
<tr>
<td>2004–2006</td>
<td>9,537</td>
</tr>
</tbody>
</table>

\(^1\)Statistically significant trend.
SOURCE: CDC/NCHS, National Health Interview Survey.

Branum et al., 2008, NCHS Data Brief
Immune Response during Food Allergy
Cells of the Adaptive Immune System: T Lymphocytes

- CD4\(^+\) T helper (Th) cells produce stimulatory and regulatory cytokines
T cell differentiation

- **Th0**
  - **Th1**: Intracellular pathogens
  - **Th2**: Helminths, nematodes
  - **Th17**: Extracellular pathogens
  - **Treg**: Prevention of autoimmunity
  - **Tfh**: B cell activation
Th0

Th1 (IFNγ)

Th2 (IL-4, IL-5, IL-13)

Th17 (IL-17A, IL-17F, IL-22, IL-21)

Treg (TGFβ, IL-10)

Tfh (IL-4, IL-21)
Peanut allergen is recognized by dendritic cells, which present the allergen to specific T cells. This results in Th2 differentiation, leading to allergen-specific B cells producing IgE and IgG antibodies. Mast cell degranulation occurs, releasing histamine, prostaglandins, leukotrienes, and cytokines like IL4, IL-5, and IL-13.

**Local**
- Swelling
- Itching
- Nausea
- Vomiting
- Diarrhea

**Systemic**
- Hives/Rash
- Respiratory distress
- Blood pressure drop
- Arrhythmia

Image from Wikimedia Commons
Antigen Processing and Presentation

- Foreign antigen
  - Antigen uptake
  - Processing
  - Peptide binds MHC
  - MHC transport vesicle
  - Presentation (surface expression) of MHC and peptide
Immune response to food allergen

- **Peanut Allergen**
- **Dendritic Cell**
- **Allergen-specific T cell**
- **Th2 differentiation**
- **Allergen-specific B cell**
- **IgE and IgG production**
- **Mast cell**

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**Mast cell degranulation**
- Histamine
- Prostaglandins
- Leukotrienes
- Cytokines
Immune response to food allergen

**Sensitization**
- Peanut Allergen
- Dendritic Cell
- Allergen-specific T cell
- Th2 differentiation
- Allergen-specific B cell
- IgE and IgG production

**Elicitation**
- **Local**
  - Swelling
  - Itching
  - Nausea
  - Vomiting
  - Diarrhea

- **Systemic**
  - Hives/Rash
  - Respiratory distress
  - Blood pressure drop
  - Arrhythmia

- Mast cell degranulation
  - Histamine
  - Prostaglandins
  - Leukotrienes
  - Cytokines

Image from Wikimedia Commons
Food allergy is normally prevented by oral tolerance

Adapted from von Boehmer et al., 2007, Journal of Experimental Medicine
GMOs: Evaluating allergenicity of novel proteins

- Sequence-based testing
- Digestion stability
- Protein body and matrix effect
- Serum testing
- Use of animal models?
Animal models of food allergy

- Atopic dog
- Neonatal swine
- Brown Norway rat
- Mouse models

Images from Wikimedia Commons
Animal Models: Neonatal Swine & Dog

**Sensitization (i.p., 3 – 5 wk): allergen + cholera tox**

- Challenge

**Sensitization (s.c., 3 – 6 mo): allergen + alum**

- Challenge

**Lactation**

1. Skin prick test
2. Allergen-specific antibodies in serum
3. PCA (Passive Cutaneous Anaphylaxis): a surrogate for IgE quantification
4. Histology
5. Endoscopy
6. Clinical symptoms

Helm, 2002; Rupa et al., 2008; Buchanan et al., 2002)

Images from Wikimedia Commons
Animal Models: Passive Cutaneous Anaphylaxis

Inject (dermal) anti-sera to target antigen (may heat inactivate)

24 - 72h

Inject (i.v.) target antigen
Optional: Evan’s blue dye

30 min

Mouse:
1. Ear thickness
2. Diameter of lesion
3. Histology

Pig:
1. Wheal & flare
2. Diameter of lesion

Images from Wikimedia Commons
Animal Models: Brown-Norway rat

1. Antigen-specific IgG and IgE in serum
2. PCA (Passive Cutaneous Anaphylaxis)
3. Gut Permeability (serum level of bystander protein)
4. Blood Pressure
5. Respiration rate
6. Clinical symptoms

Knippels et al., 1998, 1999, 2002

Images from Wikimedia Commons
**Animal models: Mouse**

**Sensitization (various routes): +/- adjuvant**

1. **3 Wk C3H mice**: Cow’s milk allergen + cholera toxin, 6 wk oral sensitization (Li et al, 1999). Similar protocol with peanut

2. **3 + 5 Wk C3H mice**: Peanut allergen + cholera toxin, 6 wk oral sensitization (Li et al, 1999)

3. **BALB/c mice**: Adjuvant-free ovalbumin, bovine serum albumin or potato protein extract by either oral gavage (daily, 42 days) or by i.p. injection (twice, 1wk apart). Dearman et al., 1999)
Animal models: Transdermal adjuvant-free sensitization mouse model

Sensitization (transdermal, 4 – 6 wk): various allergens  

- Allergen-specific IgE and IgG₁ in serum
- Decrease in body temperature
- Clinical symptoms (ranging from scratching/rubbing and edema to labored respiration and cyanosis of mucous membranes)

Developed by Dr. Venugopaul Gangur
Animal models: Adjuvant-free Transdermal Mouse model

- Known allergens induce IgE production, decrease in body temperature and other clinical symptoms of anaphylaxis
  - Hazelnut
  - Sesame
  - Cashew
  - Cow’s milk
- Foods that are not typically allergenic do not induce IgE production or anaphylactic response
  - Vanilla bean extract

Gonipeta et al., 2009, 2010; Parvataneni et al., 2009; Navuluri et al., 2006
Adjuvant-free transdermal mouse model: ovalbumin sensitization

Bleed

1 Wk

OVA

Symptoms of anaphylaxis

OVA orally

Images from Wikimedia Commons
Adjuvant-free transdermal mouse model: ovalbumin sensitization

Robust increase in OVA-specific IgE in serum after 4 wk sensitization

Clinical symptoms of anaphylaxis after oral challenge

Change in body temp = ↓1.9º C

Clinical symptoms:

- Scratching/rubbing nose & mouth
- Edema of mucosal tissues
- Decreased activity
- Labored respiration
- Unresponsive after stimulus
The food preservative tBHQ inhibits Th1 cytokine production and promotes Th2 cytokine production.

The food preservative tBHQ inhibits Th1 cytokine production and promotes Th2 cytokine production

Effect of tBHQ on food allergy – study design

- Control
- tBHQ (0.001%)

1 Wk
Bleed
1 Wk
Bleed
1 Wk
Bleed
1 Wk

OVA
OVA
OVA
OVA

OVA orally

Symptoms of anaphylaxis

Images from Wikimedia Commons
## Effect of tBHQ on food allergy – results from the pilot study

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>OVA</th>
<th>OVA + tBHQ</th>
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<tbody>
<tr>
<td><strong>IgE</strong></td>
<td>ND</td>
<td>↑</td>
<td>↑↑</td>
</tr>
<tr>
<td><strong>IgG1</strong></td>
<td>ND</td>
<td>↑</td>
<td>↑↑</td>
</tr>
<tr>
<td><strong>Body temp</strong></td>
<td>No change</td>
<td>No change</td>
<td>↓ 1.3°C</td>
</tr>
<tr>
<td><strong>Clinical score</strong></td>
<td>0</td>
<td>1.5</td>
<td>2.4</td>
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