Utility of Rodent (Mouse) Models for Evaluating Protein Allergenicity

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Plant incorporated pesticides: a simplified overview

Bt toxin perforates insect midgut and thereby protects plant.
Recalled

Heat stable, digestion resistant Bt protein (Cry9C)
Enzymatic digestion

Complete digestion (labile)

Not a good target for immune system

Semi-stable

Available for processing by antigen-presenting cells

Clinical Effects
Asthma, rhinitis, bronchospasm
Eczema, urticaria, rash, edema
Nausea, vomiting, diarrhea
Anaphylactic shock

Dong et. al, TOXICOLOGICAL SCIENCES 73, 8–16 (2003)
Small, soluble proteins: transcytosis

Larger, insoluble, or aggregated proteins: taken up by M cells

Both forms of protein: paracellular transport

Bloodstream
Hepatic portal

Liver

T cell

B cell

MAC

DC

(+/-) costimulation

(active immunity or sensitization)

lack of responding cells

plasma B cell

active immunity or sensitization

oral tolerance

active peripheral regulation

oral tolerance

lack of responding cells
ORAL TOLERANCE

“the other side of the food allergy coin”

- Normal response to orally introduced food antigens is induction of tolerance (?)
- Specifically suppresses IgE! (failure = food allergy?)
- The most likely mechanism by which children outgrow food allergies
- Tolerance induction
  - Clonal deletion of T cells
  - T cell anergy
  - Active suppression by regulatory T cells – immunosuppressive cytokines
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Hepatic portal
Liver

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(+/-) costimulation

active immunity
or sensitization

active peripheral
regulation

lack of responding cells

Oral tolerance
Tolerosomes

feed mouse

bleed mouse

centrifuge

inject new mouse

sensitizing

tolerizing 40 nm

Karlsson et al. 2001
MHC Class II restricted; enhanced by LPS

Size restricted – digested protein fragments are packaged this way

May also tolerize next animal by transplanting liver from antigen fed mouse.

Oral tolerance is linked to both digestive processes and liver function
Ways to block oral tolerance

1. inhibit digestion with antacids
2. encapsulate protein to protect it until it reaches the intestine
3. inject protein directly into the ileum
4. block hepatic portal*
5. deplete NKT cells in the liver*
6. expose very young animals*

* not sufficient for IgE production; requires direct immunization
Oral tolerance demonstrated in laboratory animals

Ovalbumin is an egg white protein widely used as a model antigen for oral tolerance induction.

IgE antibody is the most susceptible to suppression by oral tolerance!
Enterocoated beads release contents only at pH > 5

Encapsulating protein protects against digestion
Oral tolerance is no longer induced when ovalbumin is encapsulated

Also demonstrated by JG Michael 1996

still requires IP immunization
Aggregated ovalbumin does not induce oral tolerance

Chemical and heat denaturation also abrogate oral tolerance (Peng 1998).

Who feeds their kids raw eggs?

still requires IP immunization
Egg allergy

- A childhood allergy: frequently outgrown (60-80% resolve by age 5).

- Patients with IgE reactivity to pepsin-digested egg allergen are less likely to outgrow the allergy and more likely to have skin reactions.

If ovalbumin readily induces tolerance, why is it a major egg allergen?
Newborn mice lack oral tolerance

When adult mice are orally exposed to ovalbumin, they exhibit reduced responses to subsequent parenteral immunization (oral tolerance).

When newborn mice are orally exposed to ovalbumin, they exhibit enhanced responses to subsequent immunization (NO oral tolerance).

still requires IP immunization

Probable cause of increased risk of food allergy in children.
Lack of oral tolerance ≠ IgE

- Sensitization to a protein requires more than just avoiding oral tolerance
- Oral tolerance is important for tempering allergic responses
- Oral route is relevant, but the typical response in lab animals is NO response (or oral tolerance).
- Options:
  1) non-oral route
  2) oral route with adjuvant
Relevance of non-oral routes and oral exposure with adjuvant

Non-oral routes:
- dermal sensitization (UK study, V. Gangur study)
- some success in differentiating allergens from non-allergens via parenteral injection

Oral route with adjuvant (cholera toxin):
- widely accepted as a model for studying mechanisms of food allergy (peanut, cow’s milk, shrimp, etc.)
- normal route of exposure
Spectrum of food allergens

- peanut
- tropomyosin
- ovalbumin
- mustard albumin
- bovine serum albumin
- potato acid phosphatase
- HGG
- RUBISCO

Ian Kimber
Spectrum of test food allergens

Based on observed allergenicity in humans
IgE responses after subcutaneous exposure

Fold-increase over naive levels

Extract-specific IgE

- Spinach
- Egg white
- Raw peanut
- Roasted peanut

* Poor correlation with observed allergenicity
* Common problem with injected materials (contaminants)

Graph showing IgE responses after subcutaneous exposure to different extracts. The graph compares the fold-increase over naive levels for spinach, egg white, raw peanut, and roasted peanut with 60 μg s.c. and 300 μg s.c. The asterisks indicate statistical significance.
Sensitization of C3H/HeJ mice with food extracts and cholera toxin

Extracts of raw or roasted peanut, egg white, spinach, brazil nut, or turkey

1, 2, or 5 mg total protein +/- 10 μg CT

1 week

1 week

Sacrificed

Endpoints: food extract-specific IgE, IgG1, and IgG in serum
IgE responses after two oral exposures with cholera toxin

% increase over naive levels

Spinach
Egg white
Peanut
Turkey
Brazil nut

IgE
Rat basophil leukemia cell assay for IgE functionality

**Anti-roasted peanut activity**

**Anti-raw peanut activity**

**Anti-egg white activity**

**Anti-spinach activity**
IgE after four oral exposures with CT: loss of selectivity

Anti-roasted peanut IgE

Anti-egg white IgE

Anti-spinach IgE
Spectrum of test food allergens

Based on observed allergenicity in mice

- Roasted peanut
- Brazil nut
- Egg white
  (odd dose response)
- Turkey
- Spinach
Egg white induces oral tolerance, and has some sensitizing potential but unusual dose responses when administered orally with cholera toxin. Most egg white proteins are readily digested, though stable fragments remain after two hours.
Pepsin digest of egg white

<table>
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</table>
No oral tolerance to peanut

but no IgE without cholera toxin via oral route

Peanut allergy is only outgrown in 20% of patients
Why are some allergens not subject to oral tolerance?

- **Digestibility (or solubility)** – many allergens resist digestion

- **Example**: roasted peanut is not very soluble, resists digestion in vitro
Bloodstream
Hepatic portal
Liver

Small, soluble proteins:
- transcytosis

Larger, insoluble, or aggregated proteins:
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Both forms of protein:
- paracellular transport

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(+/-) costimulation

active immunity
or sensitization

lack of responding cells

Oral tolerance

active peripheral regulation
What about allergens that ARE digestible?

- Frequently cause allergy in children and don’t last into adulthood – eggs, milk
- Others cause only oral allergy syndrome in adults (local sensitization, no systemic effects) – fruit & vegetable proteins
- Cross-react with respiratory allergens (pollen); route of sensitization probably not oral!
No oral tolerance to spinach

Spinach does not induce oral tolerance, but also has very little sensitizing potential when administered orally with cholera toxin. The major spinach protein is highly digestible with no fragments remaining after 15 seconds.
No oral tolerance to Brazil nut or turkey
Manipulation of oral tolerance: peanut
Manipulation of oral tolerance: Brazil nut
Conclusions

- Digestibility likely plays a role in both the ability to serve as a target for allergic responses and to participate in tolerance when administered orally.
- More thorough analysis of the actual target proteins in each extract is required.
- Additional foods need to be examined in both models and by other laboratories for validation.
- Neonatal susceptibility factors need to be identified – adult model is not sufficient based on egg data.
- Lack of oral tolerance does not equal sensitization!
- Starting material manipulation alters outcome!
<table>
<thead>
<tr>
<th>Food</th>
<th>Sensitizing</th>
<th>Tolerizing</th>
<th>Risk</th>
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<tbody>
<tr>
<td>Peanut</td>
<td>+</td>
<td>-</td>
<td>High risk</td>
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<tr>
<td>Brazil nut</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Egg white</td>
<td>+</td>
<td>+ (*N)</td>
<td>↓</td>
</tr>
<tr>
<td>Turkey</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Spinach</td>
<td>-</td>
<td>-</td>
<td>Low risk</td>
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Thank you

- Mary Jane Selgrade
- Marsha Ward
- Liz Boykin
- Lisa Copeland
- Debbie Andrews
- David Kurtz
- Jamie DeWitt
- Yong Joo Chung
- Cherie Pucheu-Haston
- Don Doerfler
Anti-peanut IgE in BALB/c and C3H/HeJ
Stability of individual food proteins in simulated gastric fluid

Results from several studies are reported as minutes to digestion with persistence of remaining fragments in parenthesis.

<table>
<thead>
<tr>
<th>Food</th>
<th>Protein</th>
<th>Study #1</th>
<th>Study #2</th>
<th>Study #3</th>
<th>Relative stability</th>
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<td>nda</td>
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<tr>
<td></td>
<td>Ara h2</td>
<td>60</td>
<td>15 (120)</td>
<td>120 (120)</td>
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<tr>
<td>Egg white</td>
<td>Ovalbumin</td>
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<td>5</td>
<td>60 (120)</td>
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<tr>
<td></td>
<td>Ovomucoid</td>
<td>8</td>
<td>0</td>
<td>0 (5)</td>
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<tr>
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<td>Rubisco</td>
<td>0</td>
<td>0</td>
<td>nda</td>
<td>- -</td>
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</tbody>
</table>

Study #1: Astwood, J.D., et al., 1996\(^1\)
Study #2: Fu, T.J., et al., 2002\(^2\); enzyme to substrate ratio 10/1
Study #3: Fu, T.J., 2006 (personal communication); enzyme to substrate ratio 1/1
nda = no data available
Ovalbumin is not sensitizing when given orally with CT either alone or as a component of egg white. Ova induces oral tolerance.
Ovomucoid is not sensitizing when given orally with CT either alone or as a component of egg white.
MW
naive oral subcutaneous
digested undigested
digested undigested
digested undigested
MW
MW
188 98 62 49 38 28 17 14 6 3
kDa
egg white western 7-31-07
naive oral subcutaneous
MW | undigested | digested | MW | undigested | digested | MW | undigested | digested

kDa

 oral sera from 4x imm

egg white western 8-3-07

naive | subcutaneous | oral
IgE to Brazil nut and turkey after two oral exposures with CT and sodium bicarbonate
IgE to Brazil nut and turkey after two oral exposures with CT without sodium bicarbonate