
Christal Bowman / MaryJane Selgrade
Immunotoxicology Branch
Experimental Toxicology Division
NHEERL
EPA
Research Goals
(NHEERL)

- Animal model for dietary allergy (disease)
- Understand mechanisms
- Relate digestibility to allergenicity
- Identify endpoints for use in an animal screening model
- Determine potency relative to known allergens
- Identify windows of vulnerability during early development

RESEARCH & DEVELOPMENT
Building a scientific foundation for sound environmental decisions
C3H/HeJ Mouse Model of Food Allergy

PREFER ORAL EXPOSURE

Major hurdle - overcome ( & understand) oral tolerance

↑ IgE and IgG1 (allergic antibody)

Develop gut endpoints

Develop skin endpoints?

↑ Eosinophilic (allergic) inflammation & bronchial responses (lung)

Goal: Relate potency of novel protein to potent food allergen (peanut) & to non-allergen (spinach) to determine relative risk
Strategies

1. Evaluate the subcutaneous route of exposure
2. Prevent induction of tolerance with cholera toxin in oral route of exposure
3. Expose young animals before they develop tolerance mechanisms
4. Protect food antigens from digestion
5. Inhibit an enzyme involved in tolerance
6. Deplete a cell type thought to participate in oral tolerance
Subcutaneous exposure of C3H/HeJ mice

Extracts of raw or roasted peanut, egg white, or spinach

30 or 60 µg total protein in each haunch

3 weeks

2 weeks

Sacrificed

Endpoints: food extract-specific IgG, IgG1 and IgE in serum
Results of subcutaneous exposure

* indicates statistically significant increase over naïve levels
Conclusions: Subcutaneous exposure

- All food extracts elicit IgE when injected subcutaneously, whether considered allergenic or not.
- Subcutaneous exposure is probably not a useful way of distinguishing allergens from non-allergens.
- Oral route is more relevant.
Oral exposure requires overcoming oral tolerance: cholera toxin (CT)

- Works as an adjuvant to enhance the immune response to co-administered antigen.
- Built-in allergic “bias” – response elicited tends toward allergic response category.
- Enhanced IgE responses to peanut when administered orally with CT (Li, 2003).
Sensitization of C3H/HeJ mice with food extracts and cholera toxin

Extracts of raw or roasted peanut, egg white, or spinach

1 or 2 mg total protein +/- 10 µg CT

1 week

1 week

Sacrificed

Endpoints: food extract-specific IgG, IgG1 and IgE in serum
Oral exposure with cholera toxin

* indicates statistically significant increase over naïve levels
Conclusions: oral exposure with CT

- Oral exposure to raw or roasted peanut elicits IgE responses in a dose-dependent manner.
- Oral exposure to egg white elicits very little IgE, while exposure to spinach elicits almost no IgE.
- Using an oral route of exposure may allow us to distinguish allergens from non-allergens, but will probably require adjuvant (exposure to food without cholera toxin = no IgE).
- Pattern of IgE response seems to reflect the relative digestibility of these foods.
- Next: test new parameters – more exposures, expanded dose range, intermittent serum sampling, younger animals.
IgE ELISA data may be validated with RBL assay

Rat basophils armed with IgE from mouse serum respond to antigen

1. Passively sensitize rat cells with test serum
2. Add allergen
3. Transfer cell supernatant
4. Add p-nitro-phenyl-N-acetyl β-D-glucosaminide substrate
5. Stop reaction
6. Read absorbance and calculate % total release
   Total Release = Triton-X lysis
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).

Probable cause of increased risk of food allergy in children.
Exposure of neonatal C3H/HeJ mice

**OVA**
(oral by pipet or injected into stomach)

**IP**
immunization with OVA + alum

Sacrificed

Day 0-1
Birth

Day 28

Day 42

Endpoints: OVA specific IgG, IgG1 and IgE in serum
Anti-OVA IgE

IgE ng/ml

BIRTH
CTRL
IP 4 wks
ALUM
CTRL
ORAL
1 MG
ALUM
ORAL
1 MG
ALUM
INJ
1 MG
OVA
INJ
1 MG
ALUM
INJ
0.1 MG
OVA
INJ
0.1 MG
OVA

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Conclusions & next steps

- Newborn mice lack oral tolerance, but do not mount sufficient responses to measure without direct immunization and are difficult to work with. May be used for identifying susceptibility factors, but probably impractical for general testing.
- Subcutaneous exposure doesn’t distinguish allergens from non-allergens.
- Oral exposure of C3H/HeJ mouse with cholera toxin may be a good model, and appears to reflect differences in digestibility.
- Digestibility issues will be addressed with encapsulated protein studies, starting next month.
- Model will be applied to Bt toxins.
Commerically available Bt corn

- Several Bt toxin (Cry protein) producing strains currently in use
- Can we get enough for testing?
- Already used in mouse chow?