SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety

Immunotoxicology in Food and Ingredient Safety Assessment: Approaches and Case Studies

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Toxicology and Food Allergy: Case Study of tBHQ

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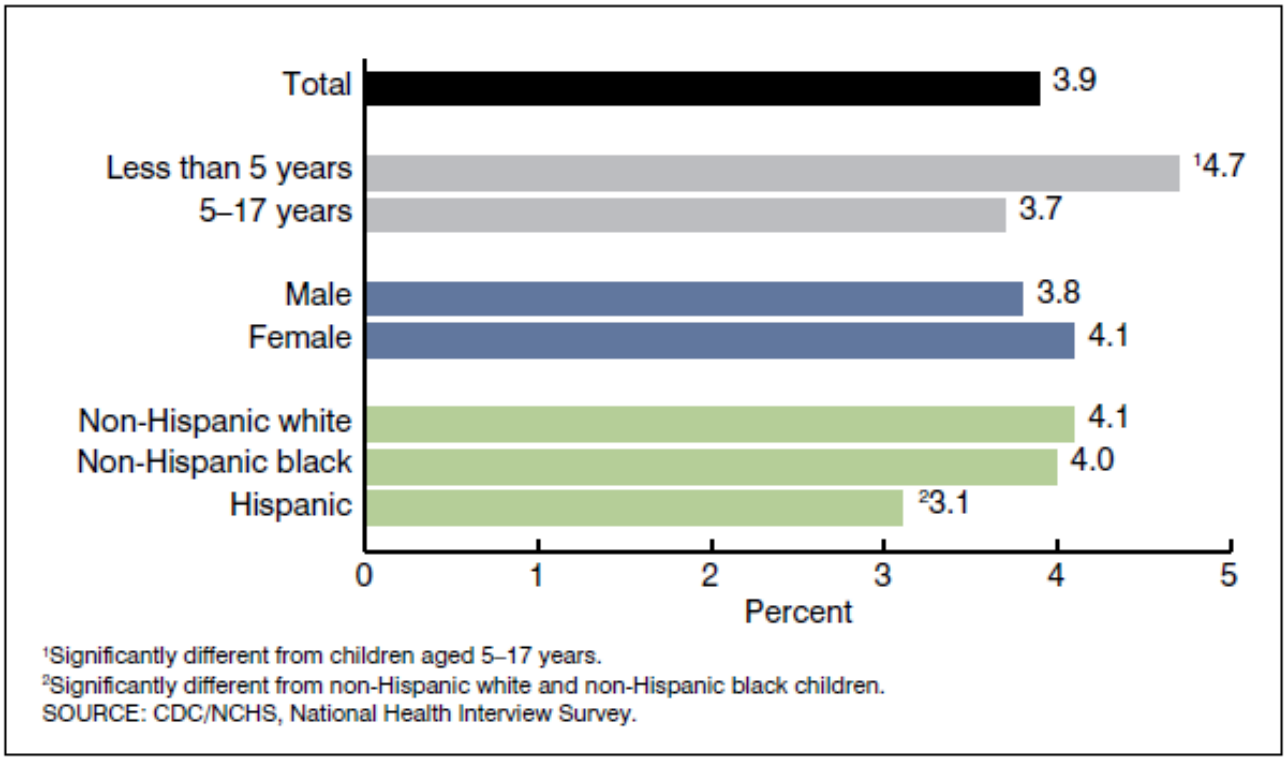
Outline

- 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



[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.

Branum et al., 2008, NCHS Data Brief

Trends in Food Allergy

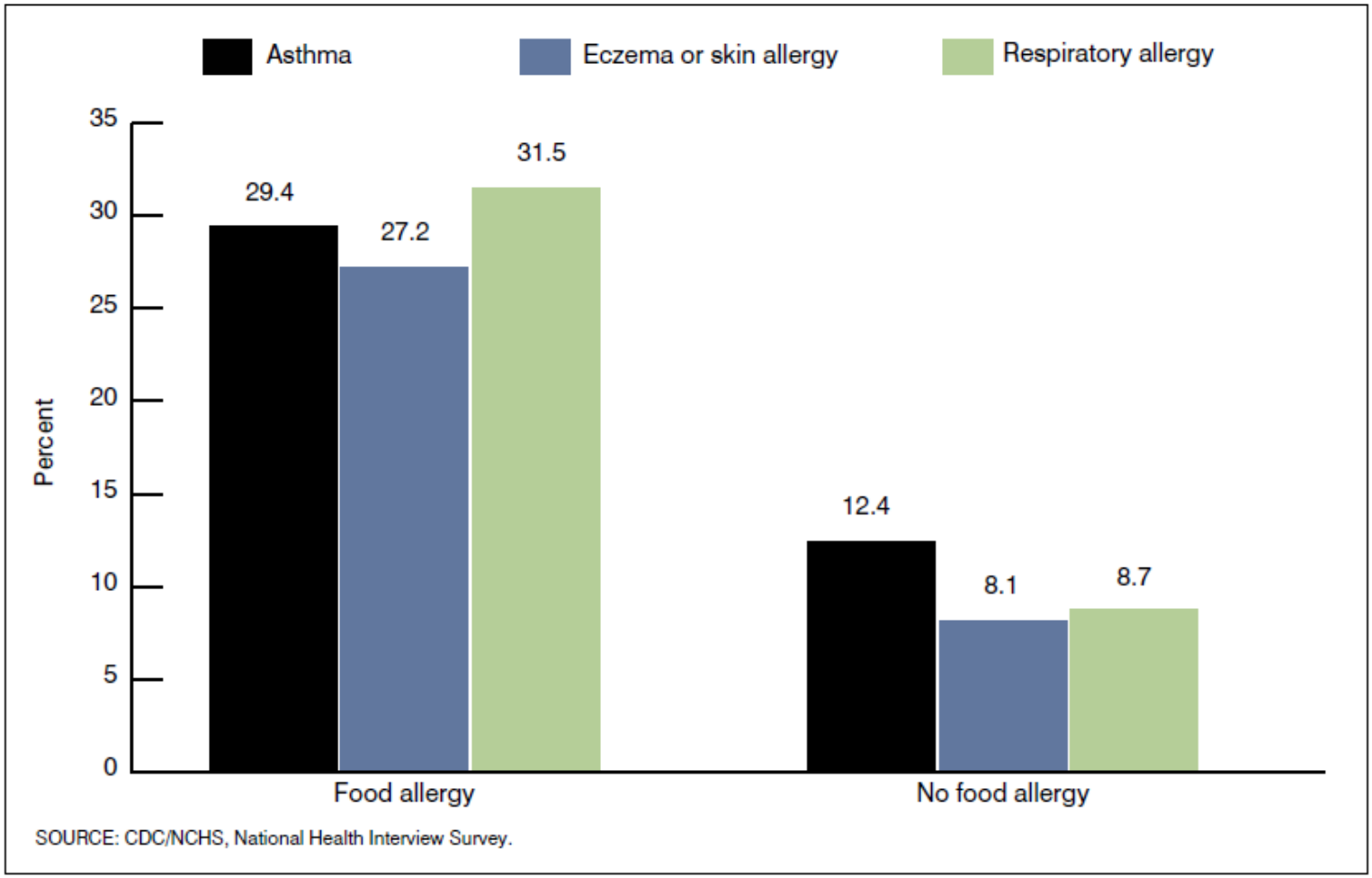
Figure 2. Percentage of children under age 18 years who had a reported food or digestive allergy in the past 12 months, by age group: United States, 1997–2007

[1]Statistically significant trend.
SOURCE: CDC/NCHS, National Health Interview Survey.

Branum et al., 2008, NCHS Data Brief

Trends in Food Allergy

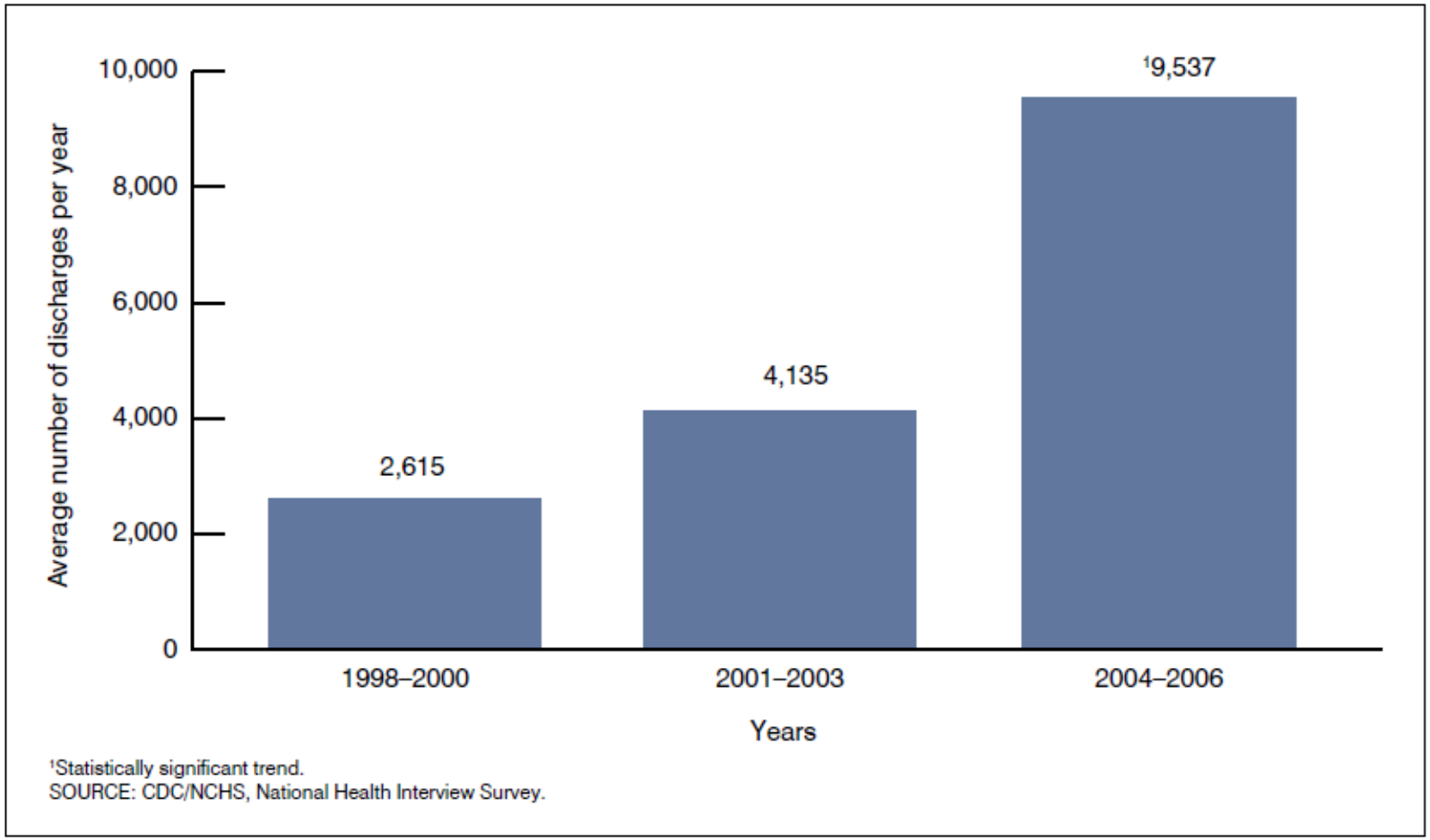
Figure 3. Percentage of children under age 18 years with asthma or other reported allergic conditions in the previous 12 months, by reported food allergy status: United States, 2007



Branum et al., 2008, NCHS Data Brief

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

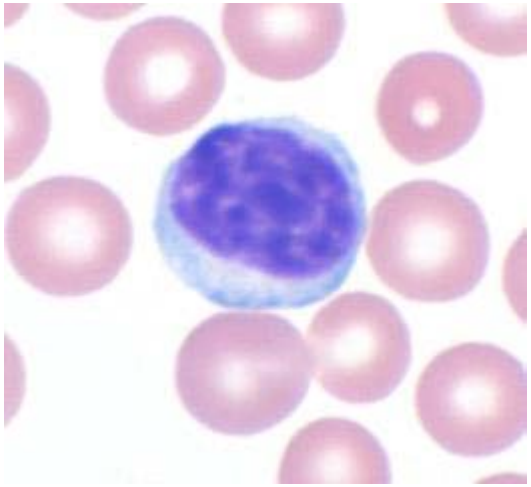


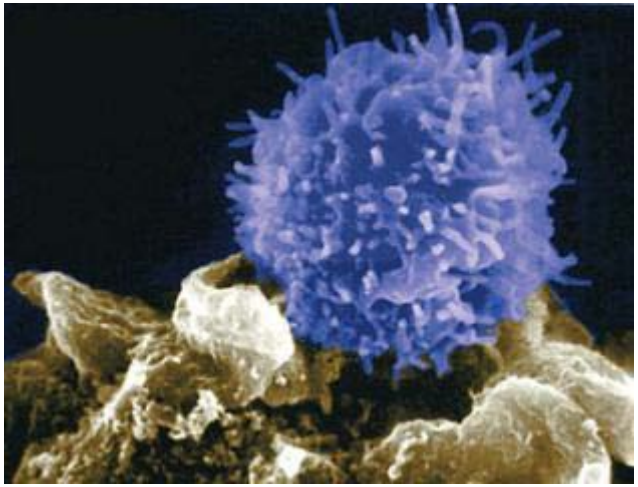
[1]Statistically significant trend.
SOURCE: CDC/NCHS, National Health Interview Survey.

Branum et al., 2008, NCHS Data Brief

Immune Response during Food Allergy

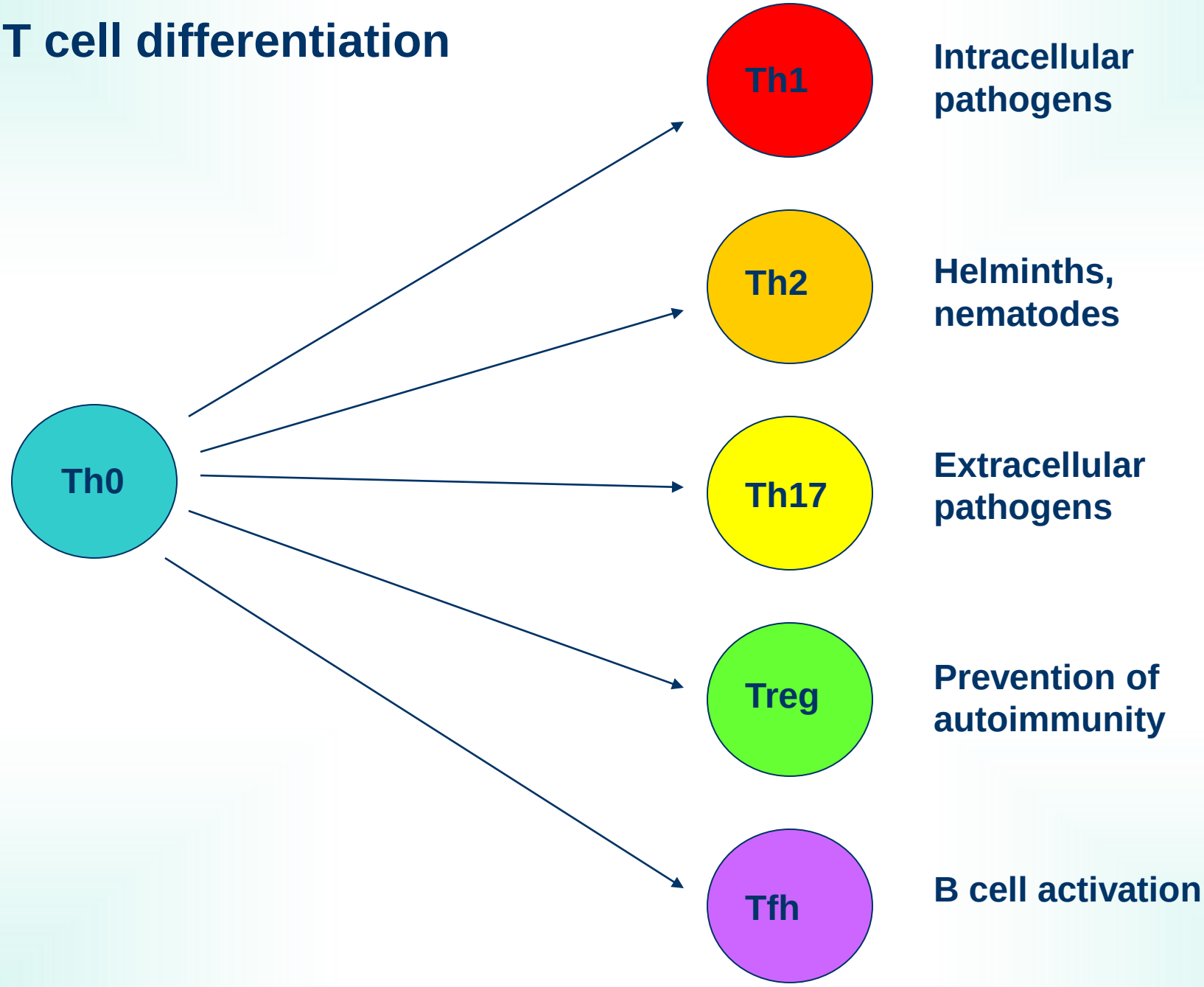
Cells of the Adaptive Immune System: T Lymphocytes

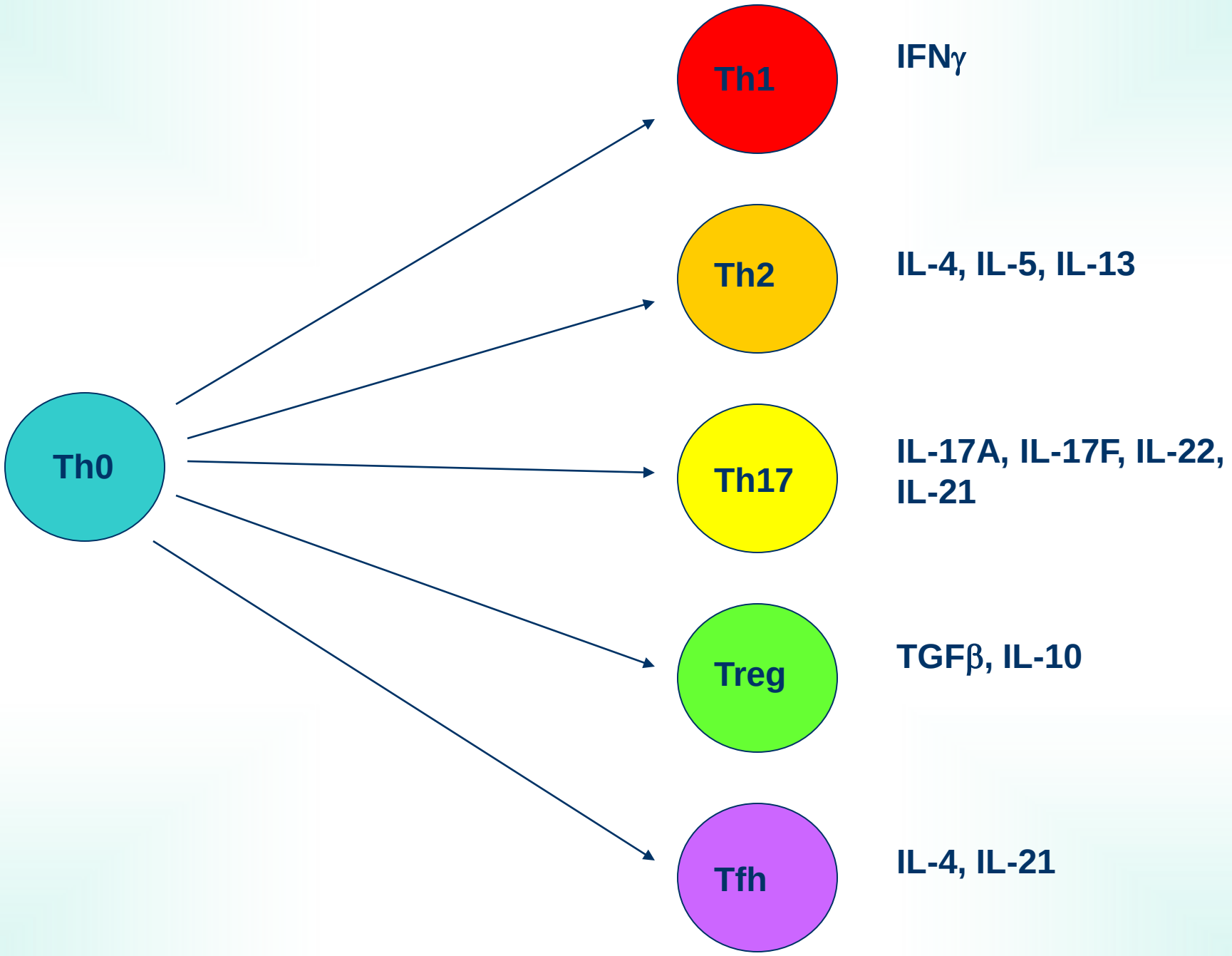




- **$CD4^+$ T helper (Th) cells produce stimulatory and regulatory cytokines**

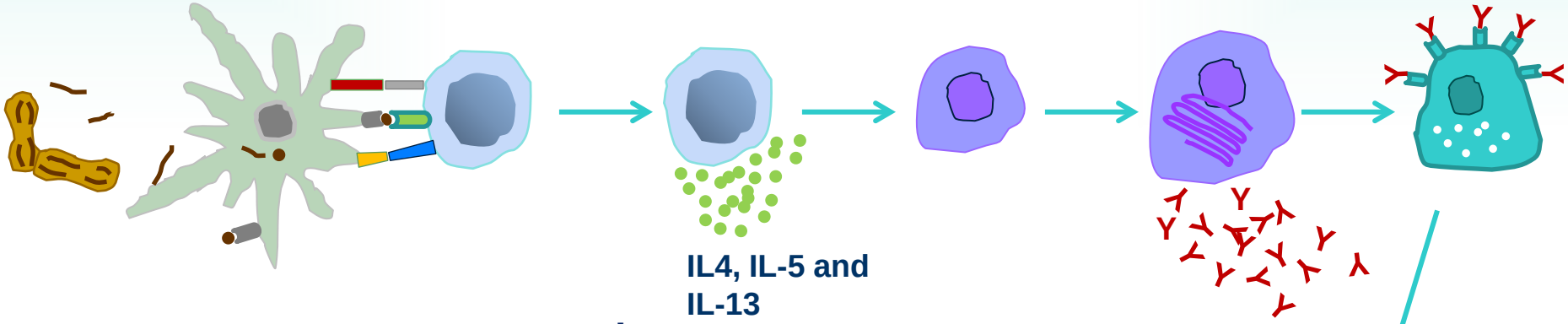
T cell differentiation



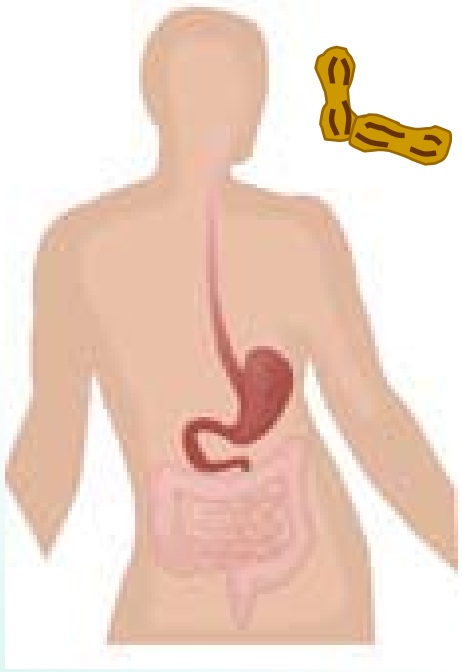

Th0
Th1
IFNγ
Th2
IL-4, IL-5, IL-13
Th17
IL-17A, IL-17F, IL-22, IL-21
Treg
TGFβ, IL-10
Tfh
IL-4, IL-21

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

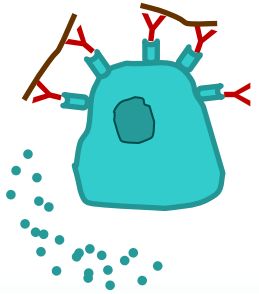


IL4, IL-5 and IL-13



Local
Swelling
Itching
Nausea
Vomiting
Diarrhea

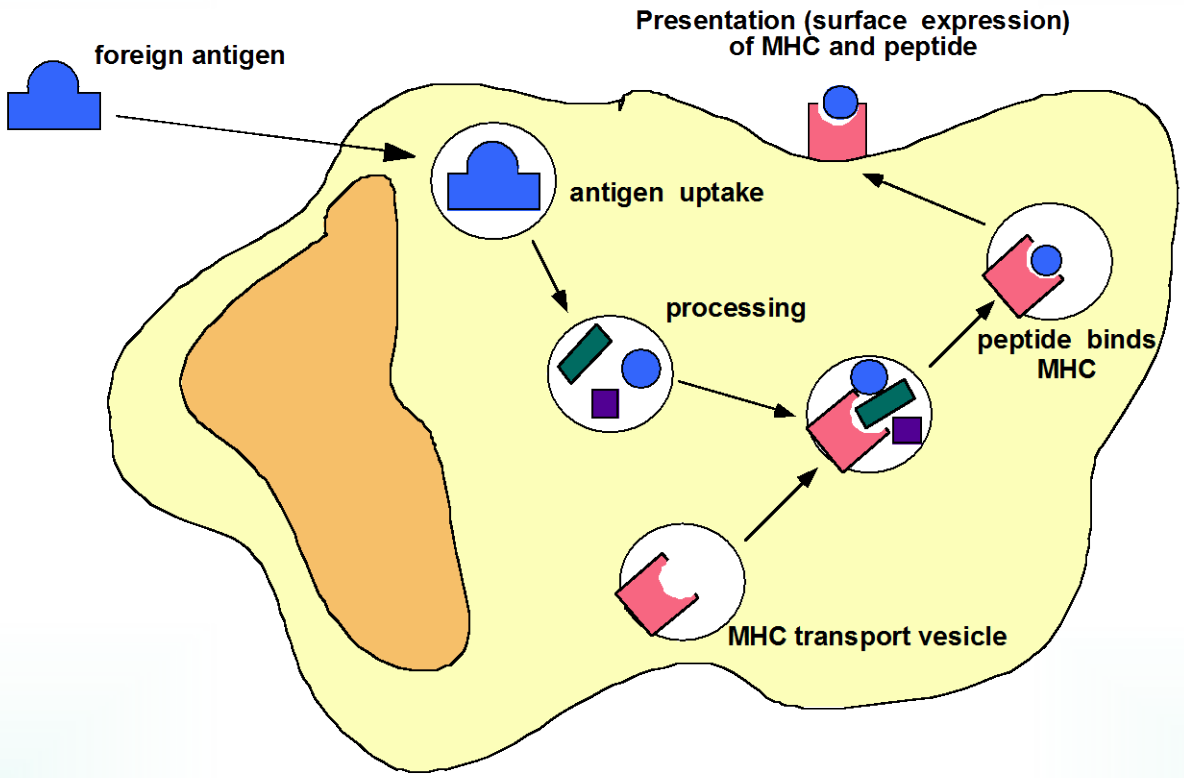
Systemic
Hives/Rash
Respiratory distress
Blood pressure drop
Arrhythmia



Mast cell degranulation
Histamine
Prostaglandins
Leukotrienes
Cytokines

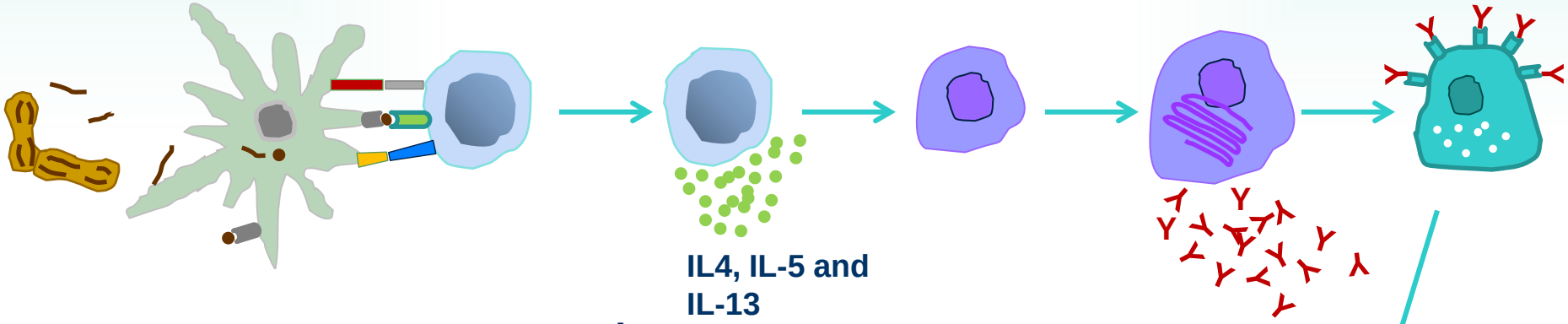
Image from Wikimedia Commons

Antigen Processing and Presentation



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



IL4, IL-5 and IL-13

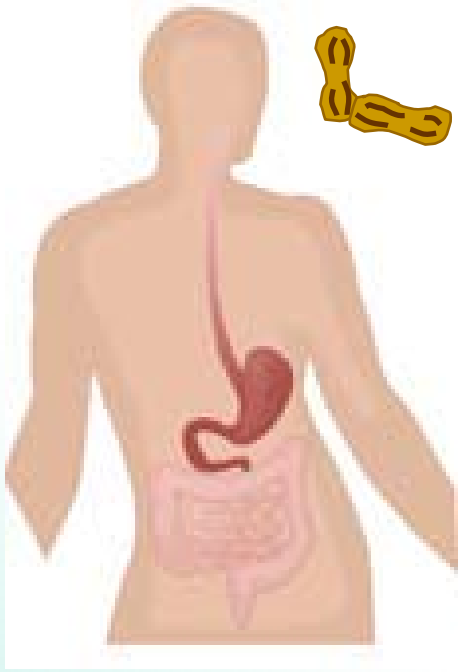


Image from Wikimedia Commons

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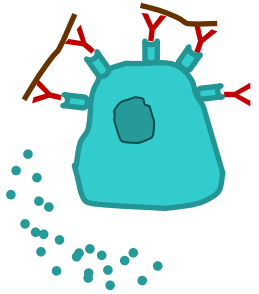
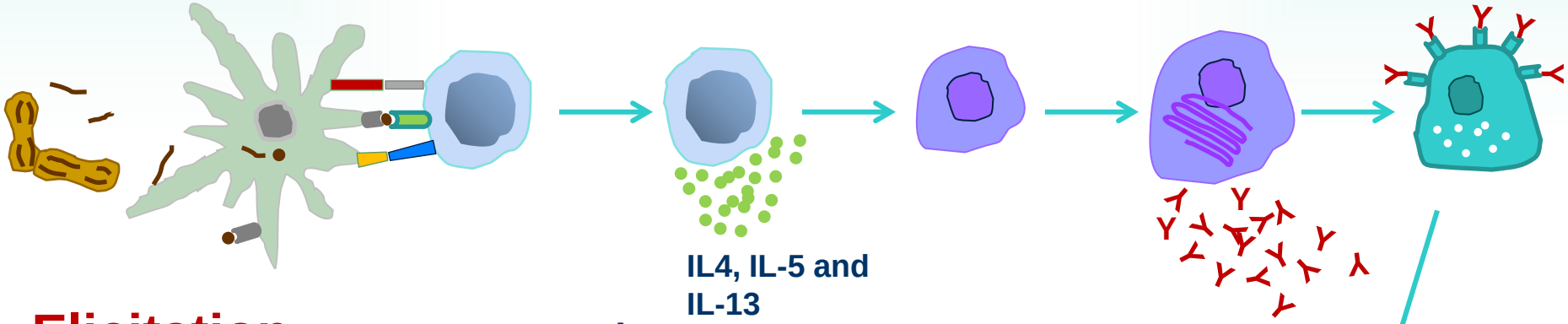


Mast cell degranulation
Histamine
Prostaglandins
Leukotrienes
Cytokines

Immune response to food allergen

Sensitization

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



IL4, IL-5 and IL-13

Elicitation

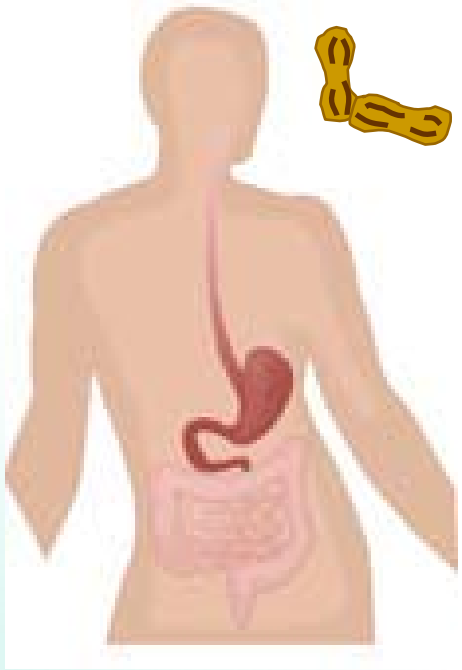


Image from Wikimedia Commons

Local
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Nausea
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Systemic
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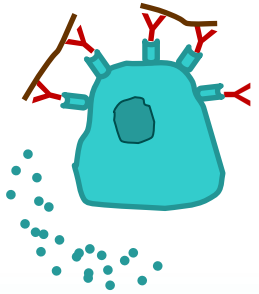
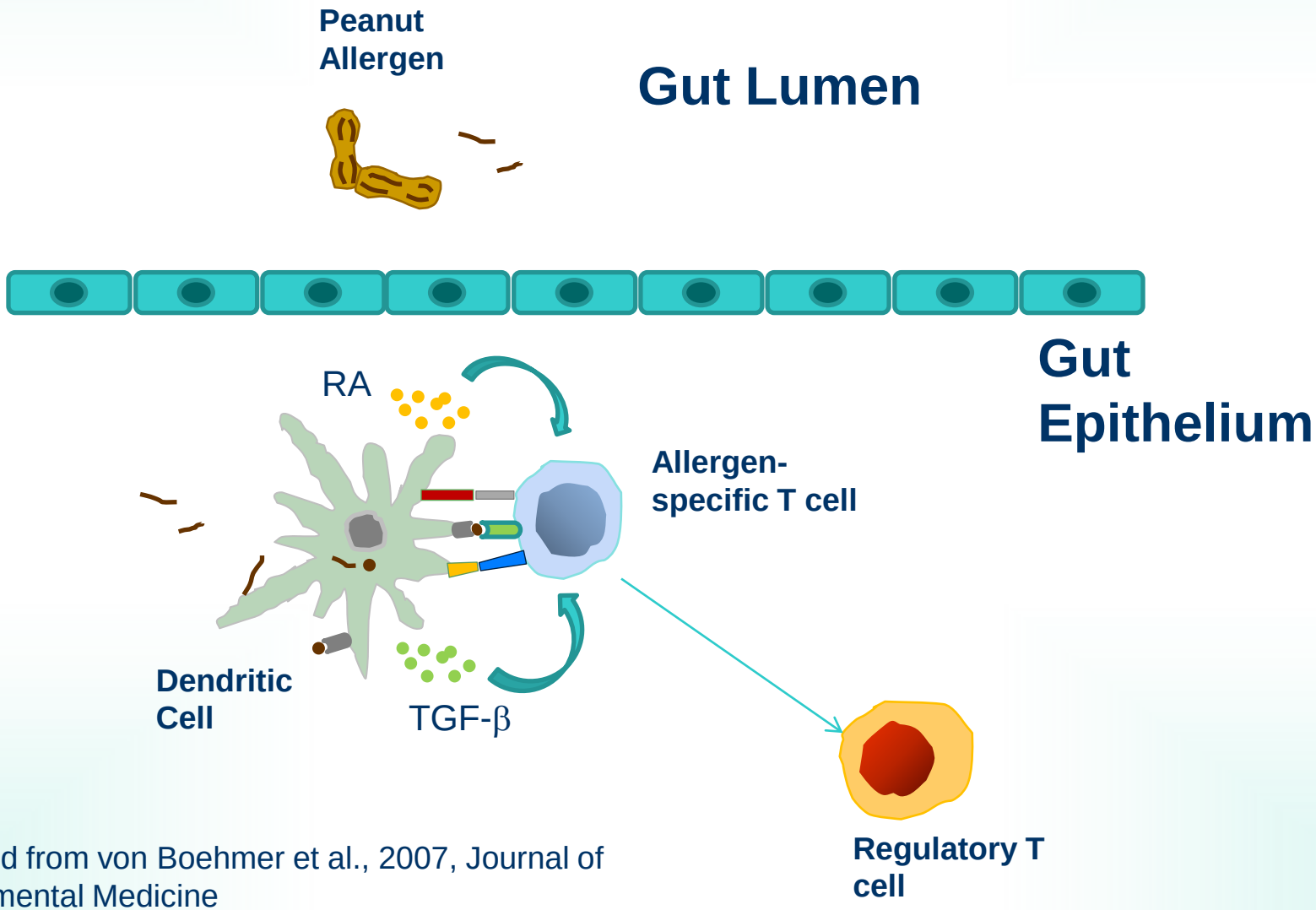


Mast cell degranulation
Histamine
Prostaglandins
Leukotrienes
Cytokines

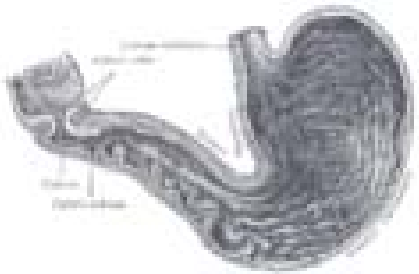
Food allergy is normally prevented by oral tolerance

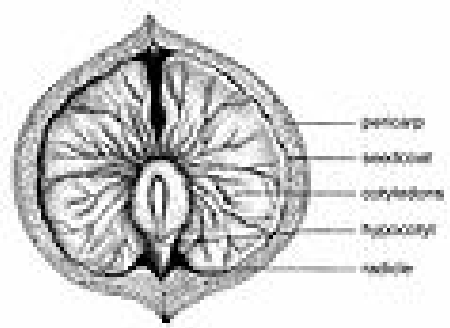


Adapted from von Boehmer et al., 2007, Journal of Experimental Medicine

GMOs: Evaluating allergenicity of novel proteins

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





Images from Wikimedia Commons

Animal models of food allergy

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

Images from Wikimedia Commons

Animal Models: Neonatal Swine & Dog

Lactation (3 wk)

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

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

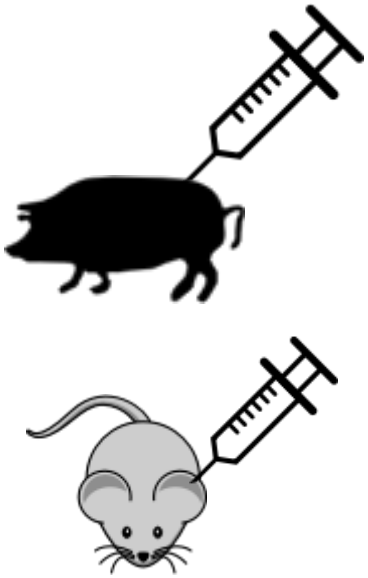
Lactation

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

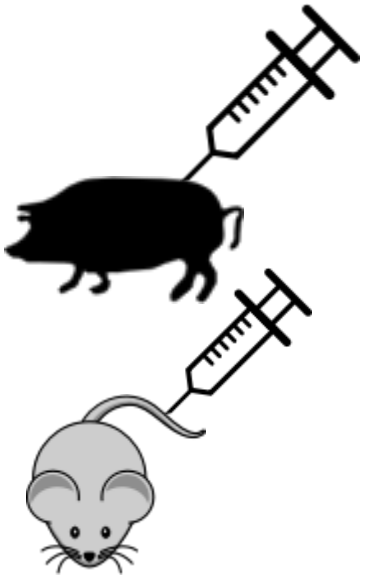
Helm, 2002; Rupa et al., 2008; Buchanan et al., 2002)

Images from Wikimedia Commons

Animal Models: Passive Cutaneous Anaphylaxis



24 - 72h



30 min

Mouse:

1. **Ear thickness**
2. **Diameter of lesion**
3. **Histology**

Pig:

1. **Wheal & flare**
2. **Diameter of lesion**

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

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

Images from Wikimedia Commons

Animal Models: Brown-Norway rat

Sensitization (daily, oral, 6 wk): allergen | **Challenge**

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

Minority of animals

Knippels et al., 1998, 1999, 2002

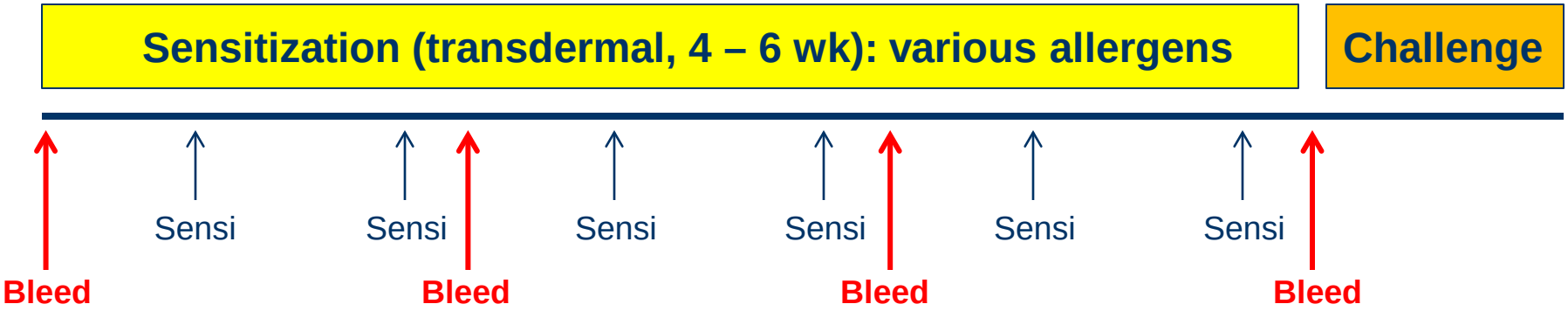
Images from Wikimedia Commons

Animal models: Mouse

Sensitization (various routes): +/- adjuvant	Challenge

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



1. Allergen-specific IgE and IgG_1 in serum
2. Decrease in body temperature
3. Clinical symptoms (ranging from scratching/rubbing and edema to labored respiration and cyanosis of mucous membranes)

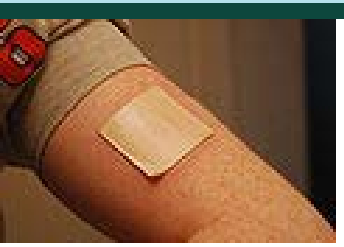
Developed by Dr. Venugopaul Gangur

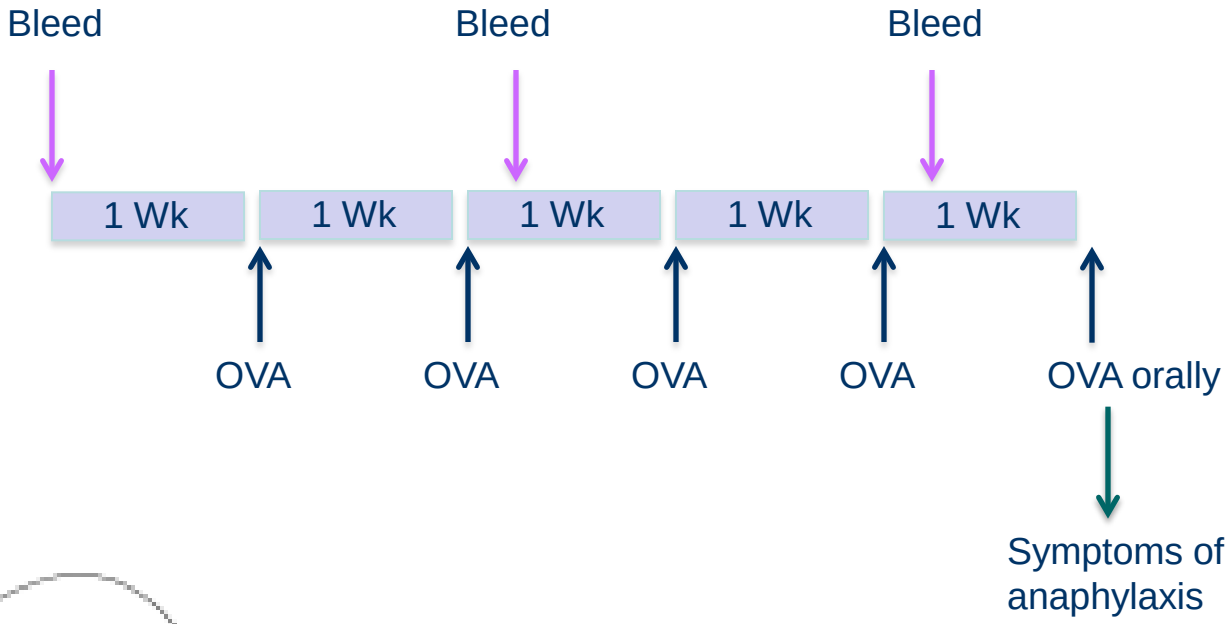
Animal models: Adjuvant-free Transdermal Mouse model

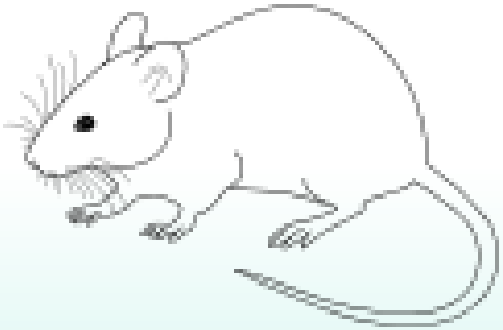
- Known allergens induce IgE production, decrease in body temperature and other clinical symptoms of anaphylaxis
  - Hazelnut
  - Sesame
  - Cashew
  - Cow's milk
- Foods that are not typically allergenic do not induce IgE production or anaphylactic response
  - Vanilla bean extract

Gonipeta et al., 2009, 2010; Parvataneni et al., 2009; Navuluri et al., 2006

Adjuvant-free transdermal mouse model: ovalbumin sensitization







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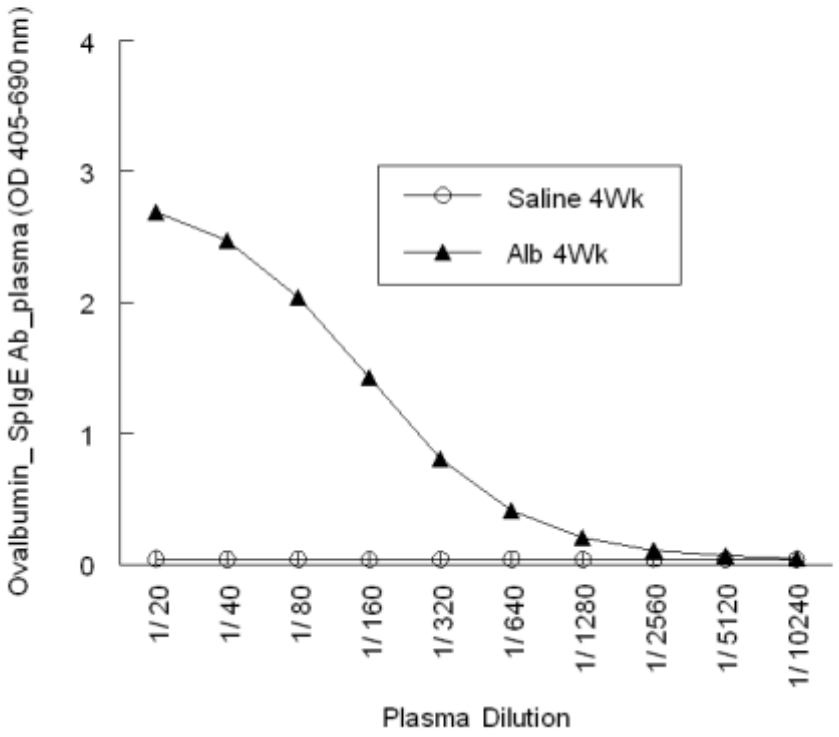
Adjuvant-free transdermal mouse model: ovalbumin sensitization

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



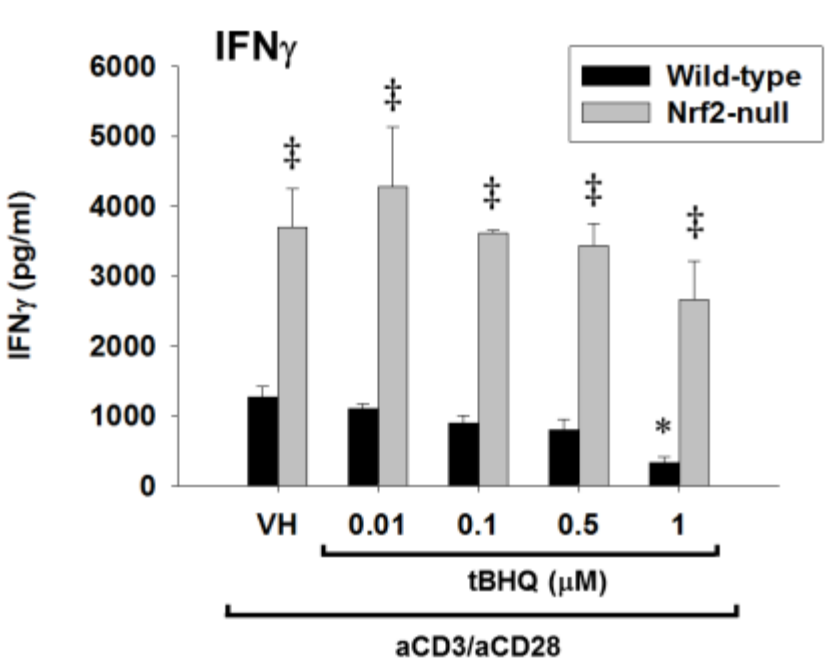
Clinical symptoms of anaphylaxis after oral challenge

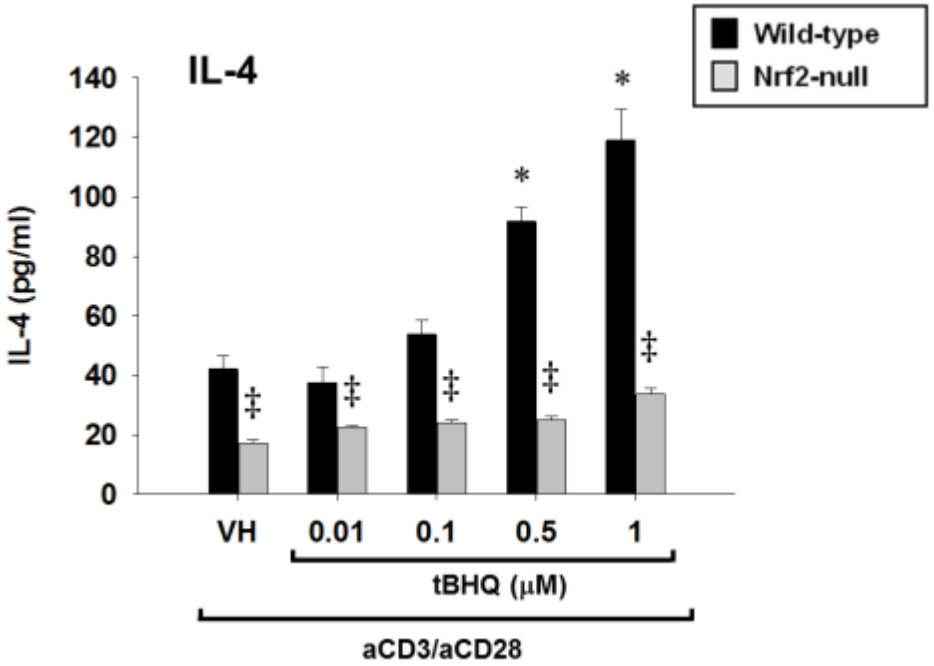
Change in body temp = ↓1.9º C

<u>Clinical symptoms</u>:

Scratching/rubbing nose & mouth
Edema of mucosal tissues
Decreased activity
Labored respiration
Unresponsive after stimulus

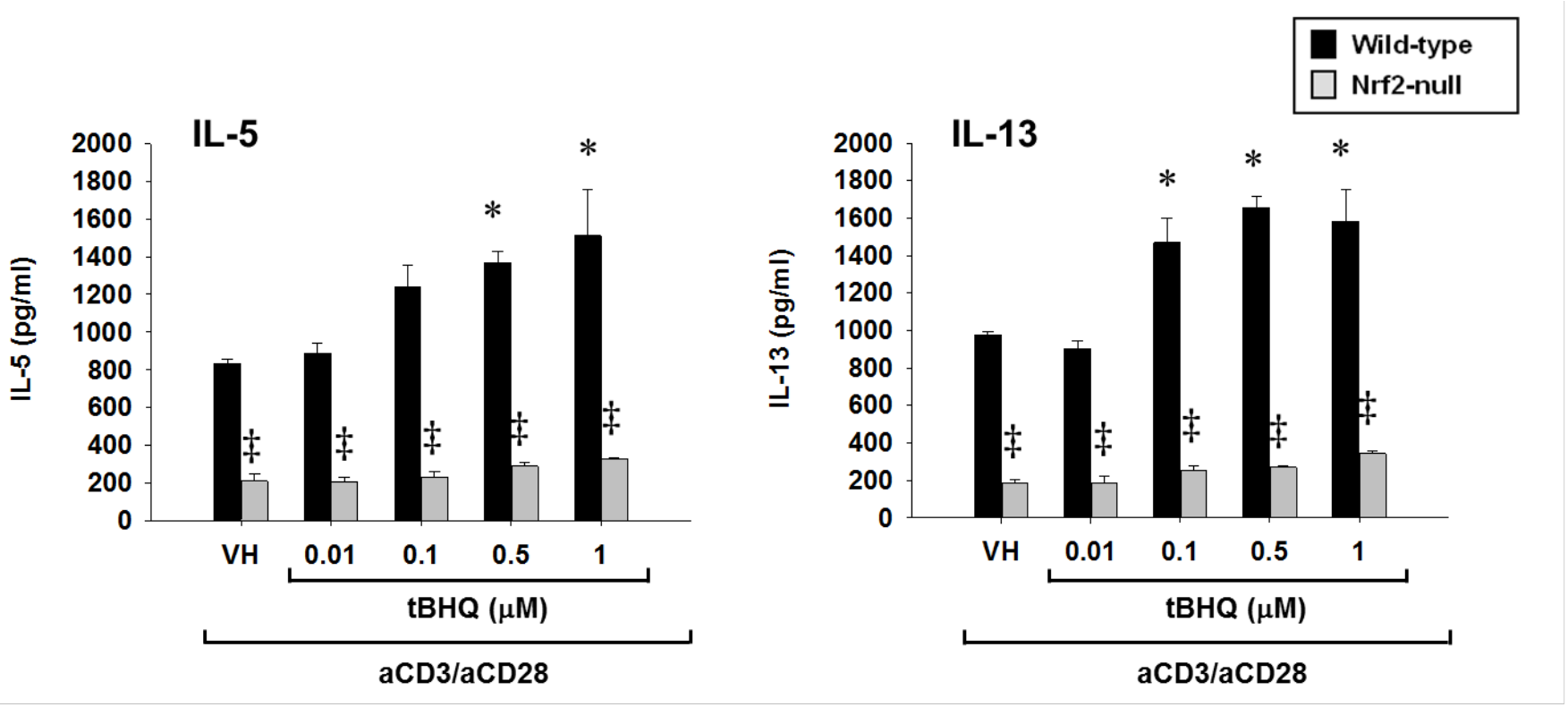
The food preservative tBHQ inhibits Th1 cytokine production and promotes Th2 cytokine production





Rockwell et al., 2012, Journal of Immunology, 188:1630-7. Copyright 2012. The American Association of Immunologists, Inc.

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

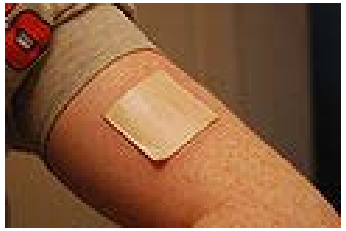


Rockwell et al., 2012, Journal of Immunology, 188:1630-7. Copyright 2012. The American Association of Immunologists, Inc.

Effect of tBHQ on food allergy– study design

Control

tBHQ (0.001%)

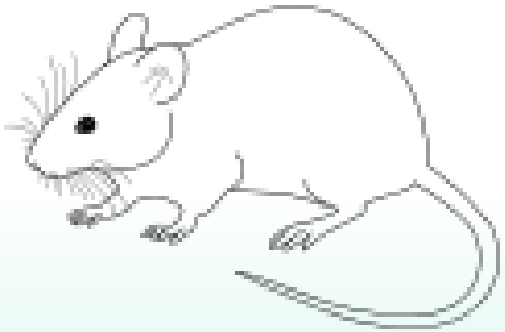


Bleed Bleed Bleed

1 Wk | 1 Wk | 1 Wk | 1 Wk | 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

	Saline	OVA	OVA + tBHQ
IgE	ND	↑	↑↑
IgG1	ND	↑	↑↑
Body temp	No change	No change	↓ 1.3°C
Clinical score	0	1.5	2.4

Acknowledgments

- Rockwell Lab
  - Heather Dover
  - Jenna Bursley
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  - Alex Turley
  - Joe Zagorski
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  - Copple Lab
- Dr. Venu Gangur
  - Tina Ortiz
  - Radhakrishna Para
  - Babu Gonipeta
- Dr. Jim Pestka