Utility of Rodent (Mouse) Models for Evaluating Protein Allergenicity

Christal C. Bowman
MaryJane K. Selgrade

US Environmental Protection Agency
Immunotoxicology Branch
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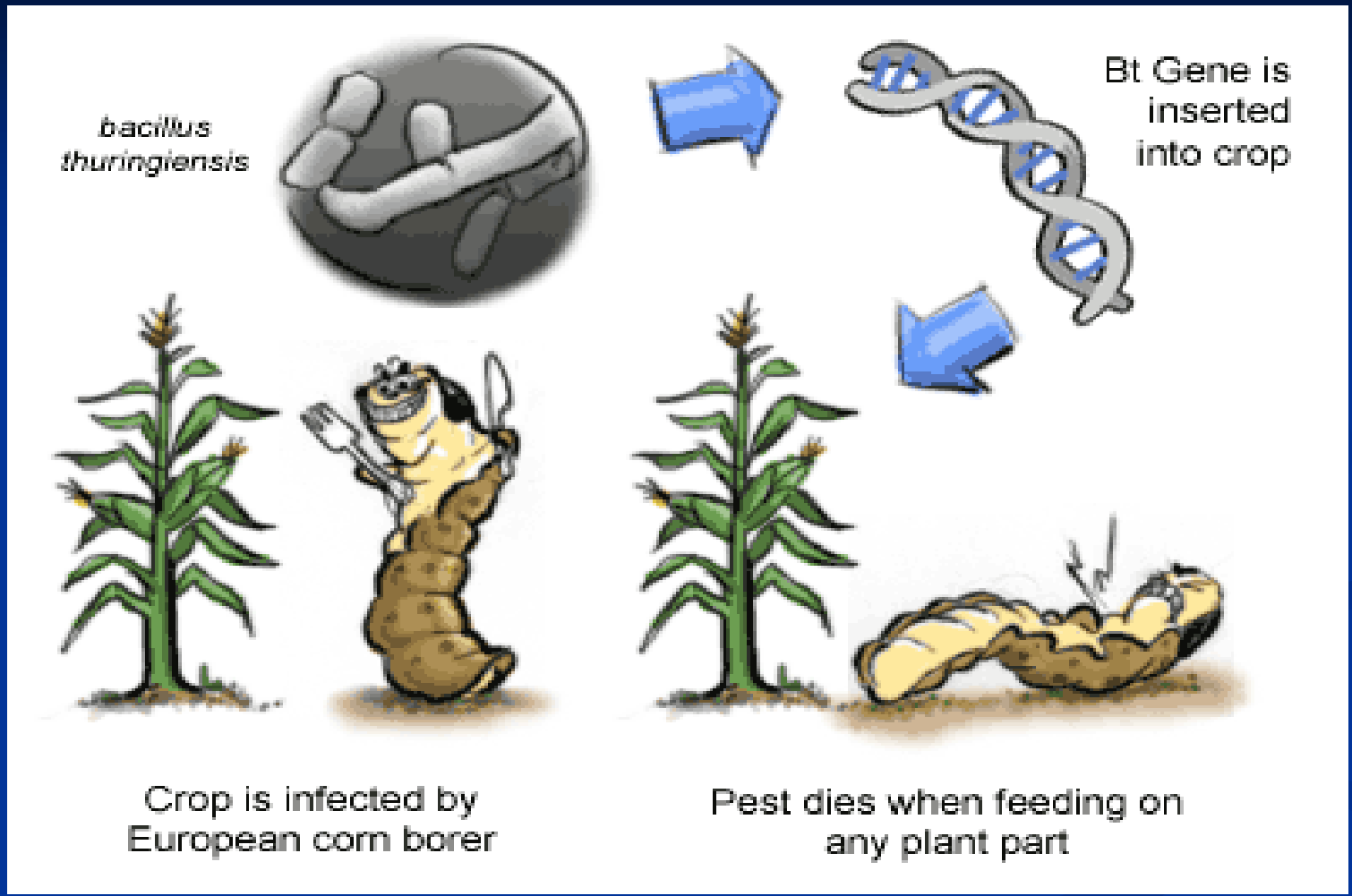
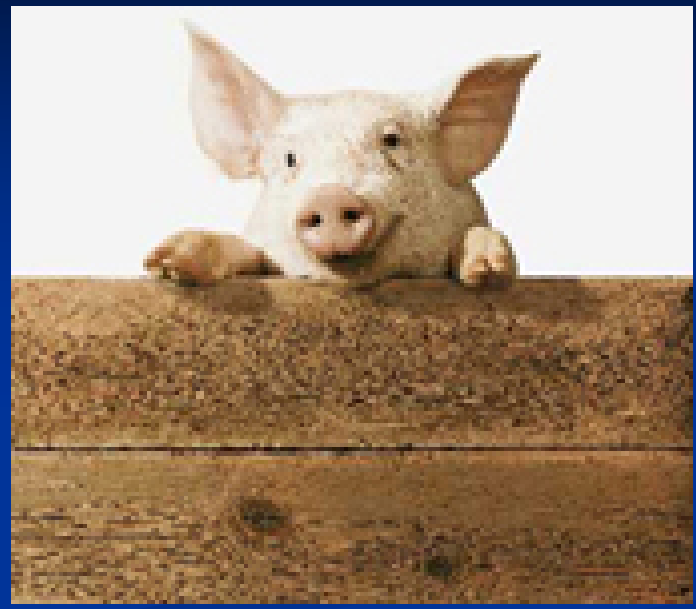
Plant incorporated pesticides: a simplified overview



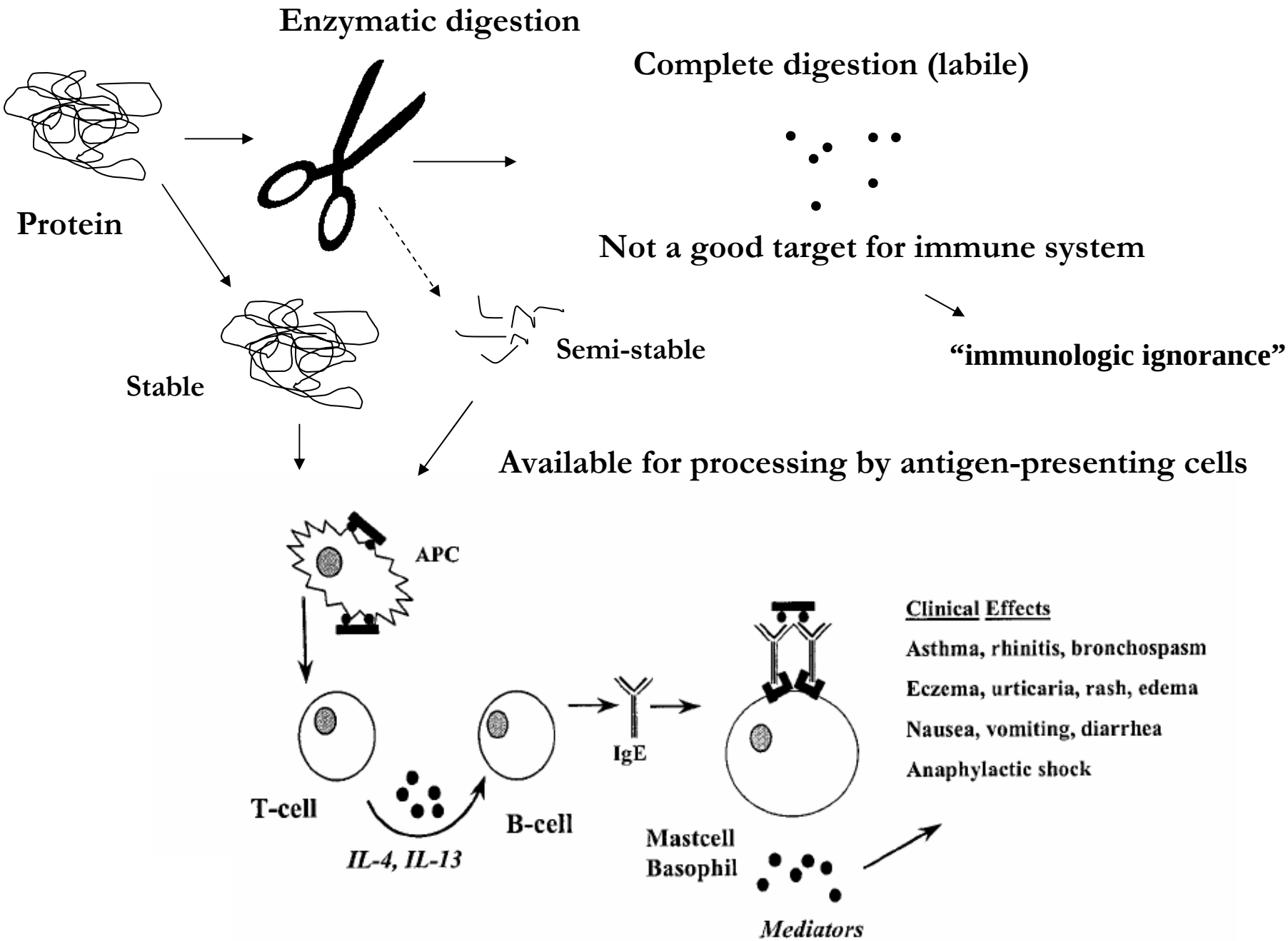
Jiang Long/Illustrator "The Science Creative Quarterly" (www.scq.ubc.ca)

Bt toxin perforates insect midgut and thereby protects plant.

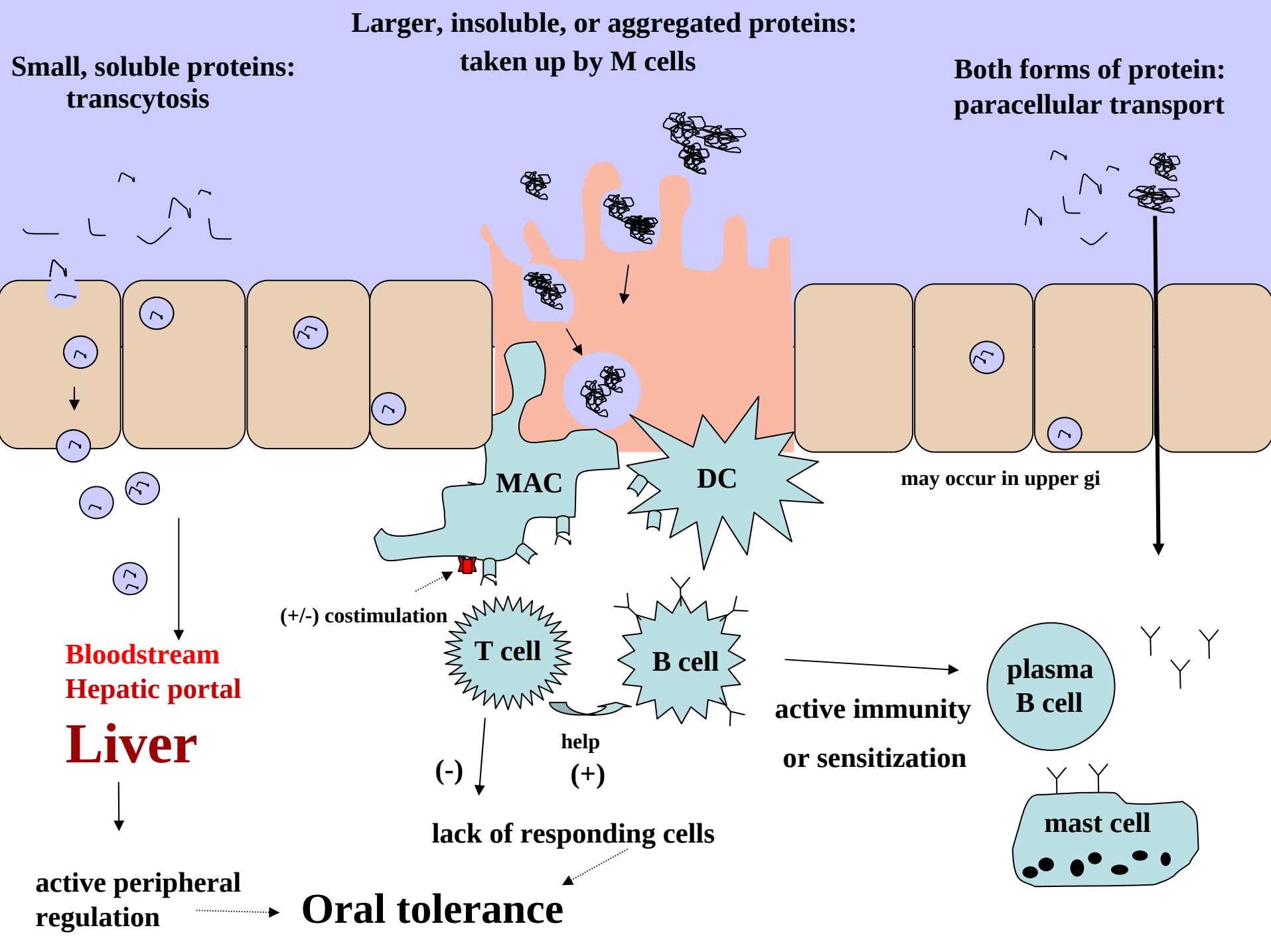


Heat stable, digestion resistant Bt protein (Cry9C)

Recalled



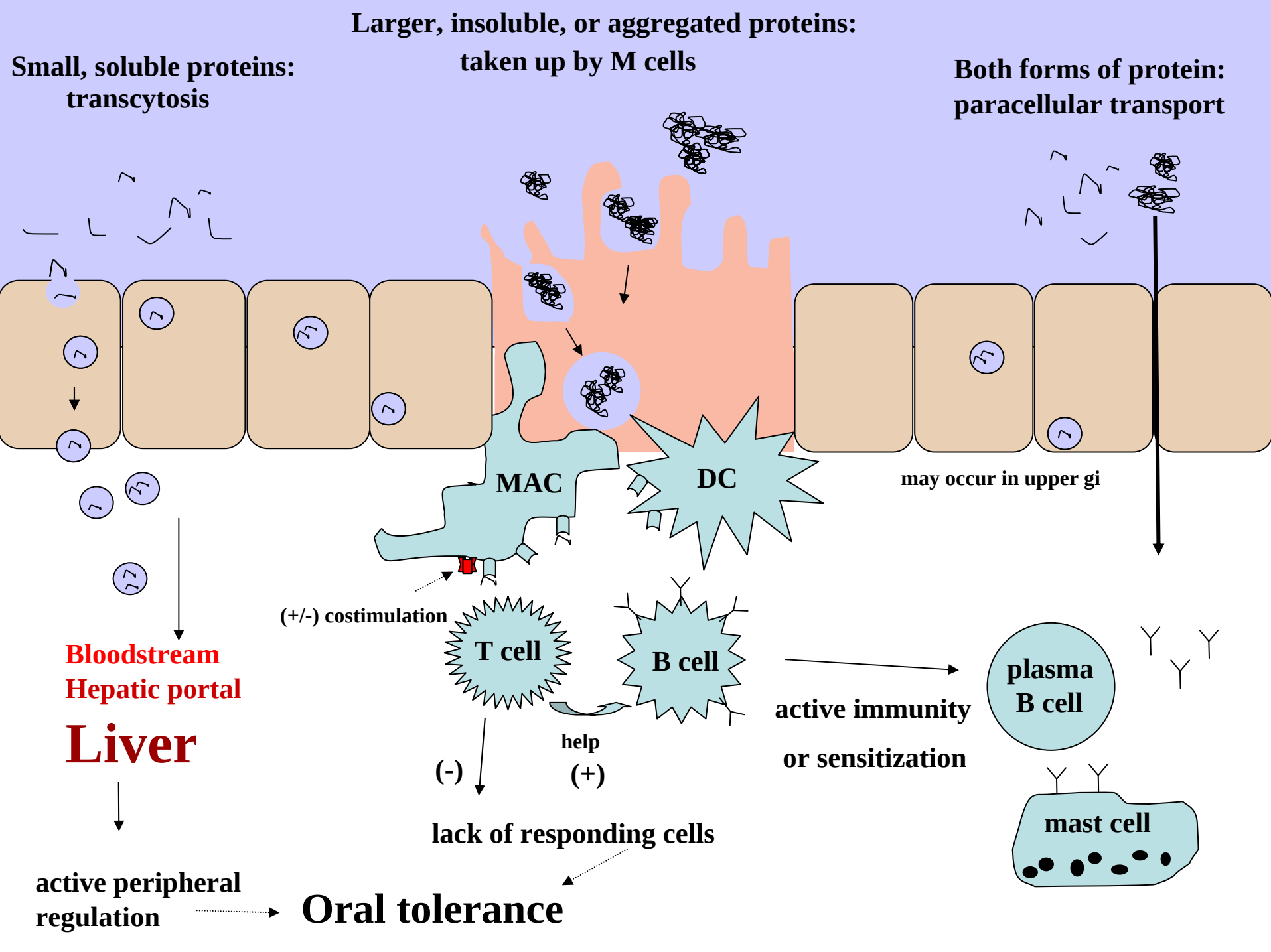
Dong et. al, TOXICOLOGICAL SCIENCES 73, 8–16 (2003)


Larger, insoluble, or aggregated proteins:
taken up by M cells
Small, soluble proteins:
transcytosis
Both forms of protein:
paracellular transport
MAC
DC
may occur in upper gi
(+/-) costimulation
T cell
B cell
Bloodstream
Hepatic portal
Liver
help
(-)
(+)
active immunity
or sensitization
plasma
B cell
mast cell
lack of responding cells
active peripheral
regulation
Oral tolerance

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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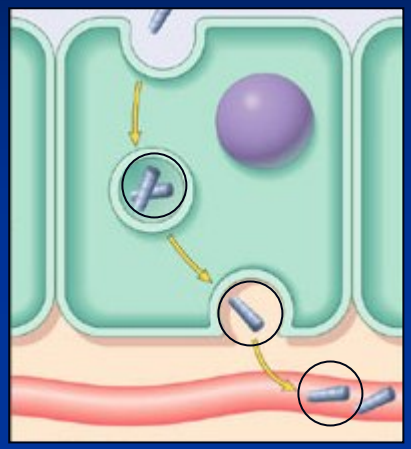
Oral tolerance

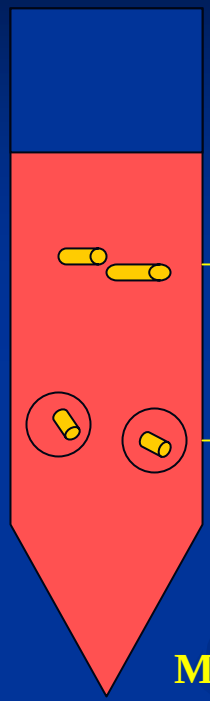
Tolerosomes

feed mouse



bleed mouse

centrifuge



inject new mouse

sensitizing

tolerizing 40 nm

Karlsson et al. 2001

MHC Class II restricted; enhanced by LPS

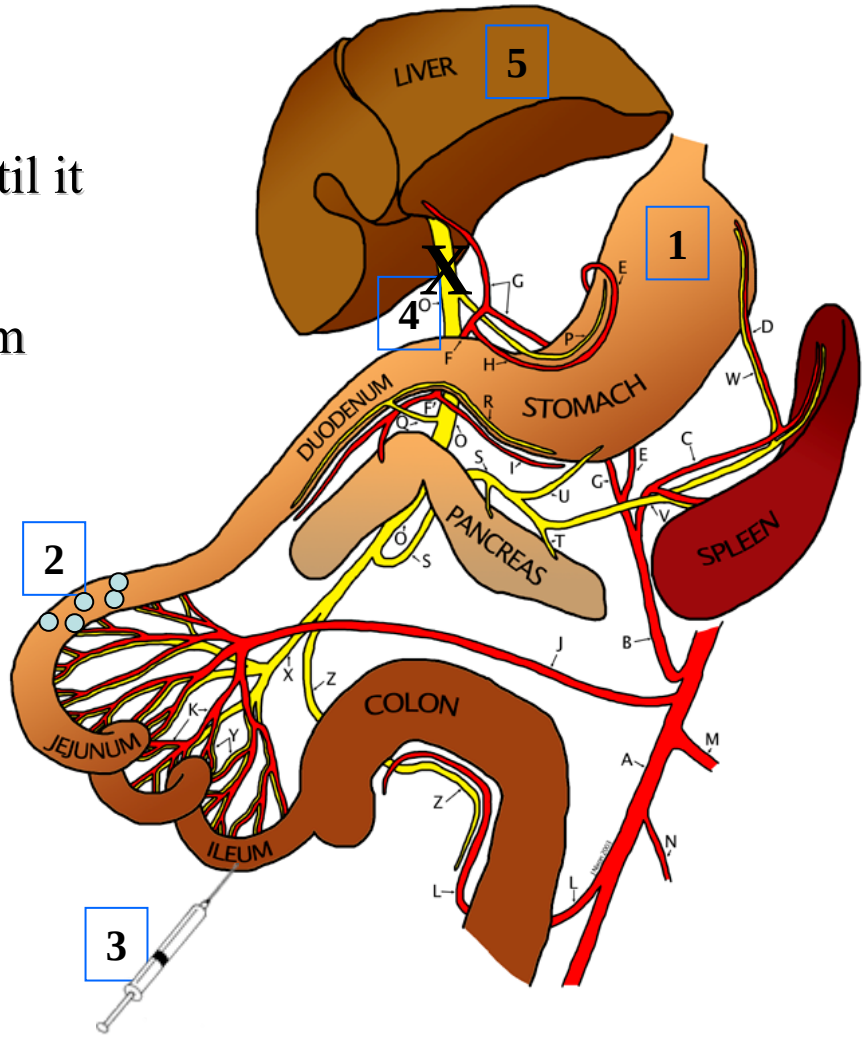
Size restricted – digested protein fragments are packaged this way

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

Oral tolerance is linked to both digestive processes and liver function

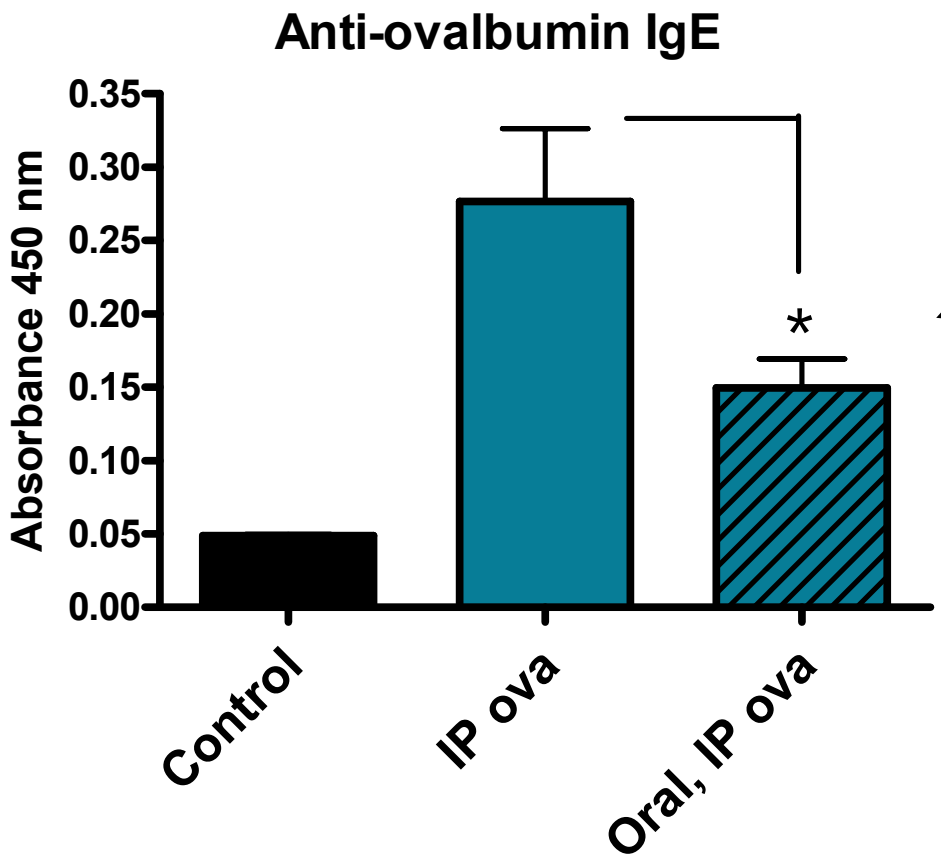
Ways to block oral tolerance

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**

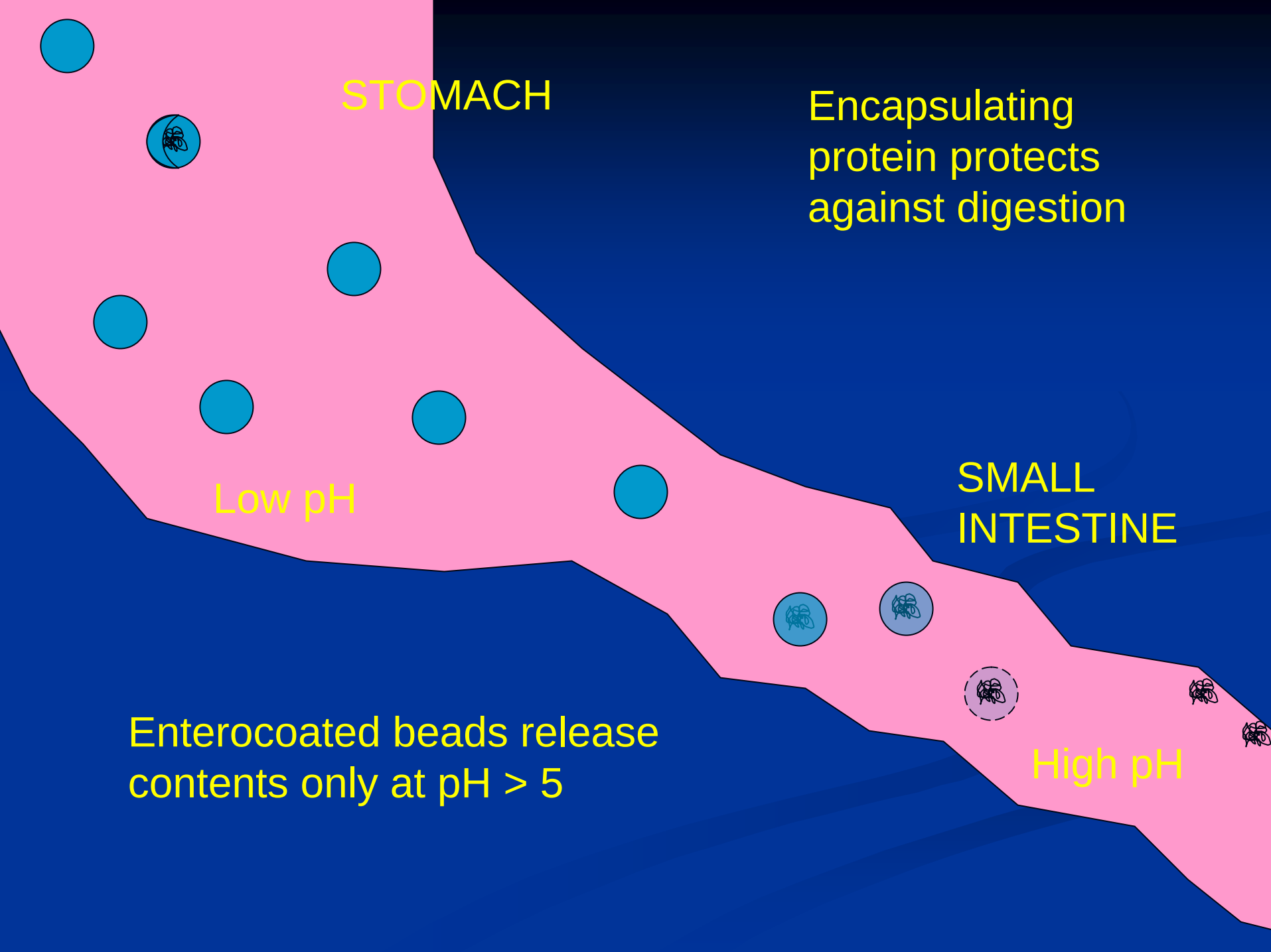
Oral tolerance demonstrated in laboratory animals



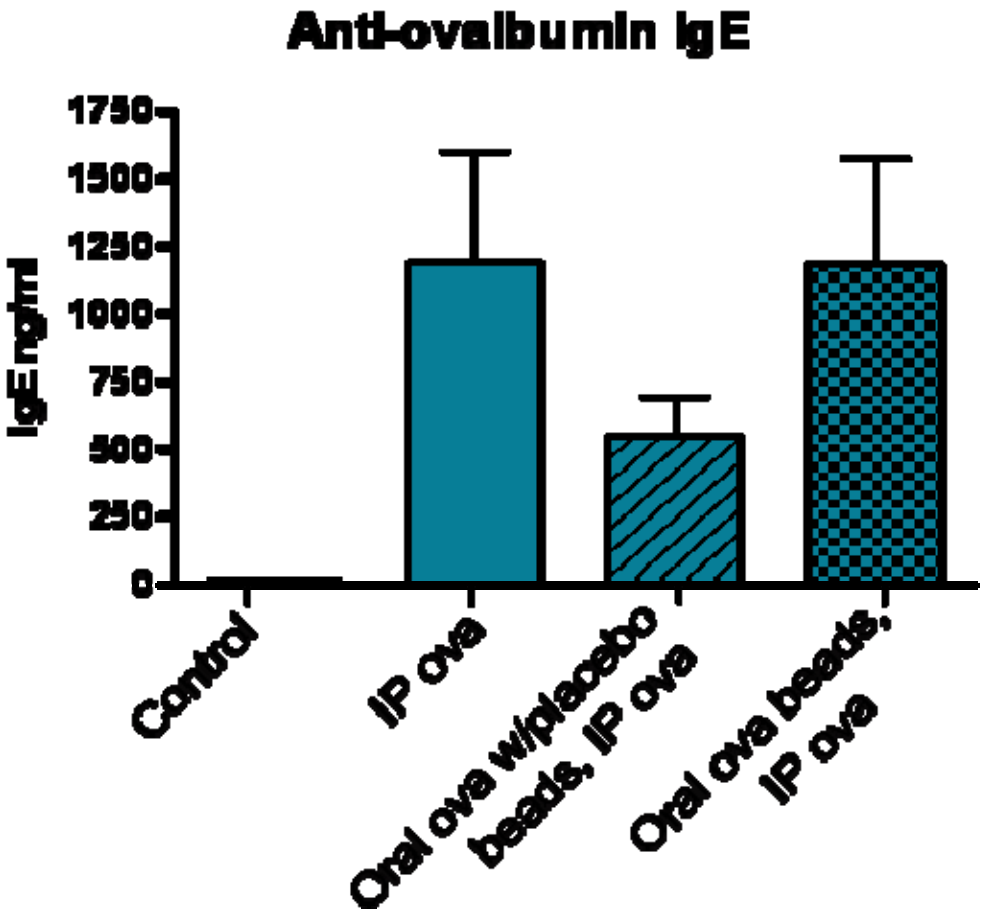
response is reduced by prior oral exposure

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!


STOMACH
Encapsulating protein protects against digestion
Low pH
SMALL INTESTINE
Enterocoated beads release contents only at pH > 5
High pH

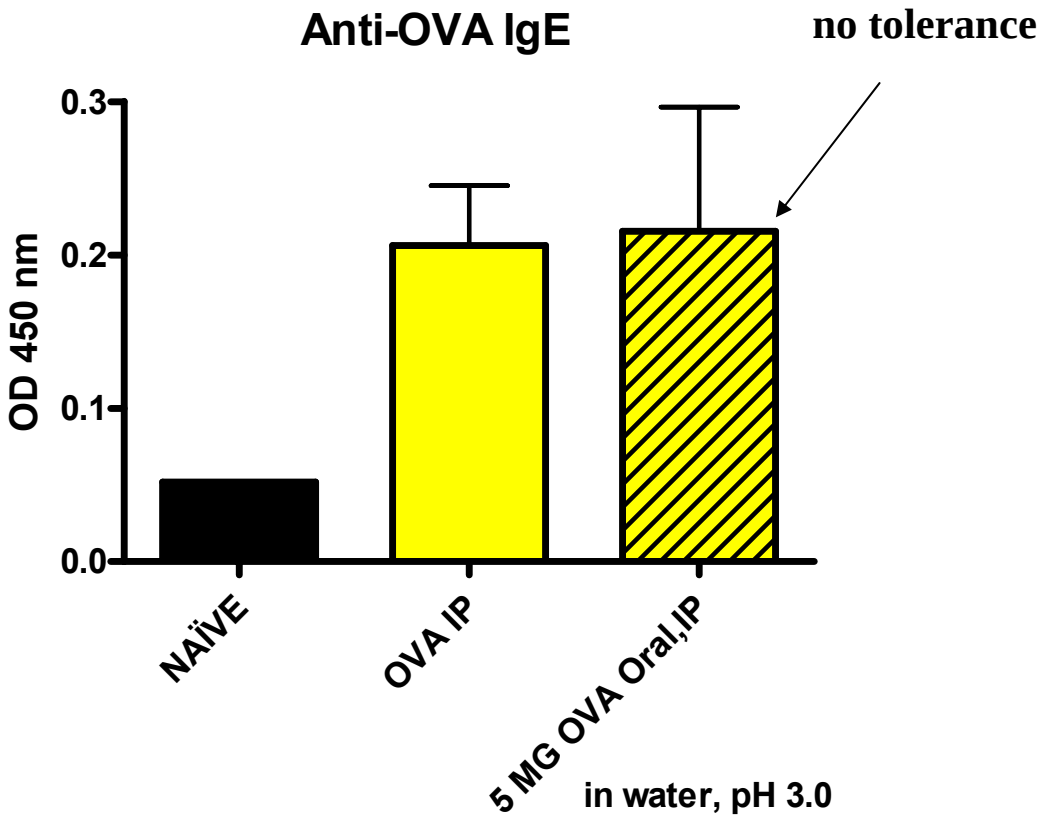
Oral tolerance is no longer induced when ovalbumin is encapsulated



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

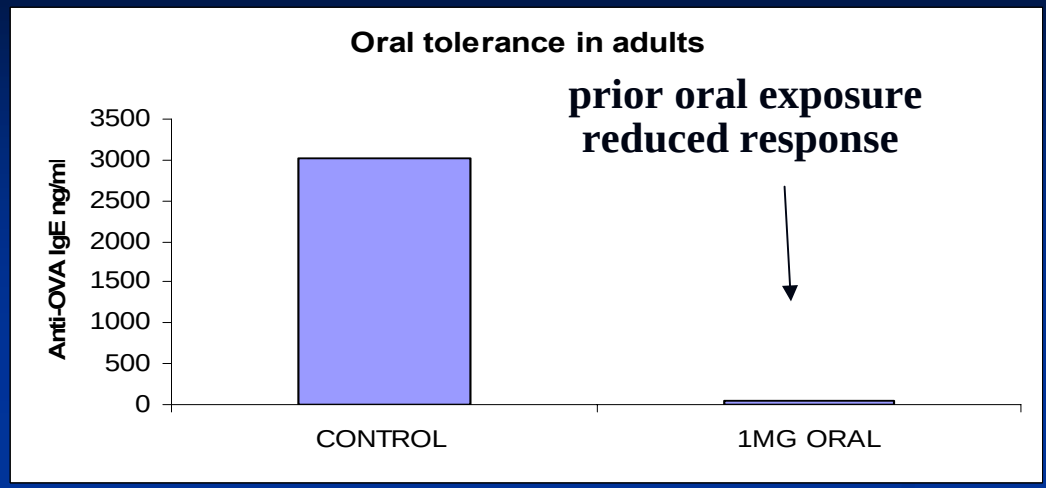
Egg allergy

- 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.

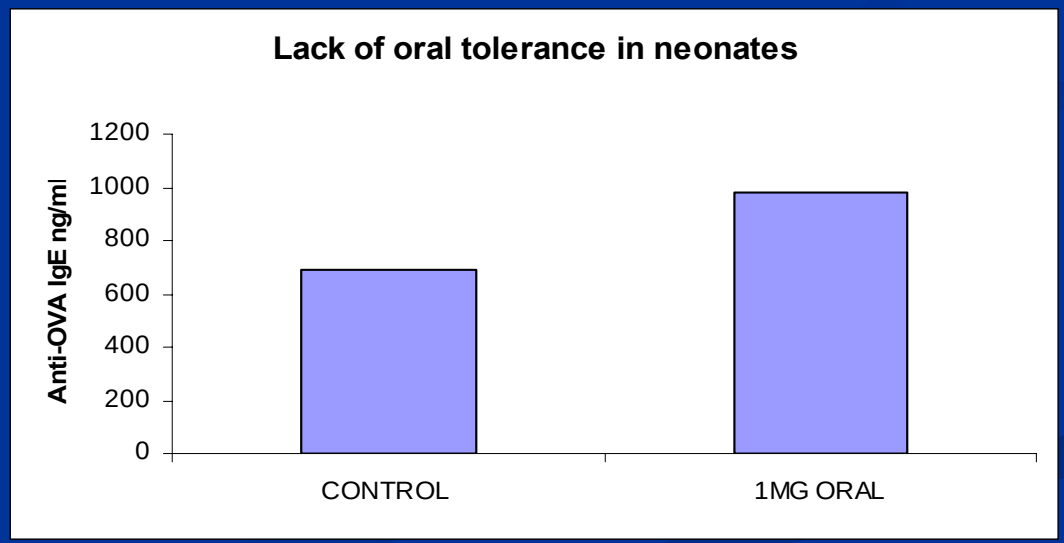
If ovalbumin readily induces tolerance, why is it a major egg allergen?

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

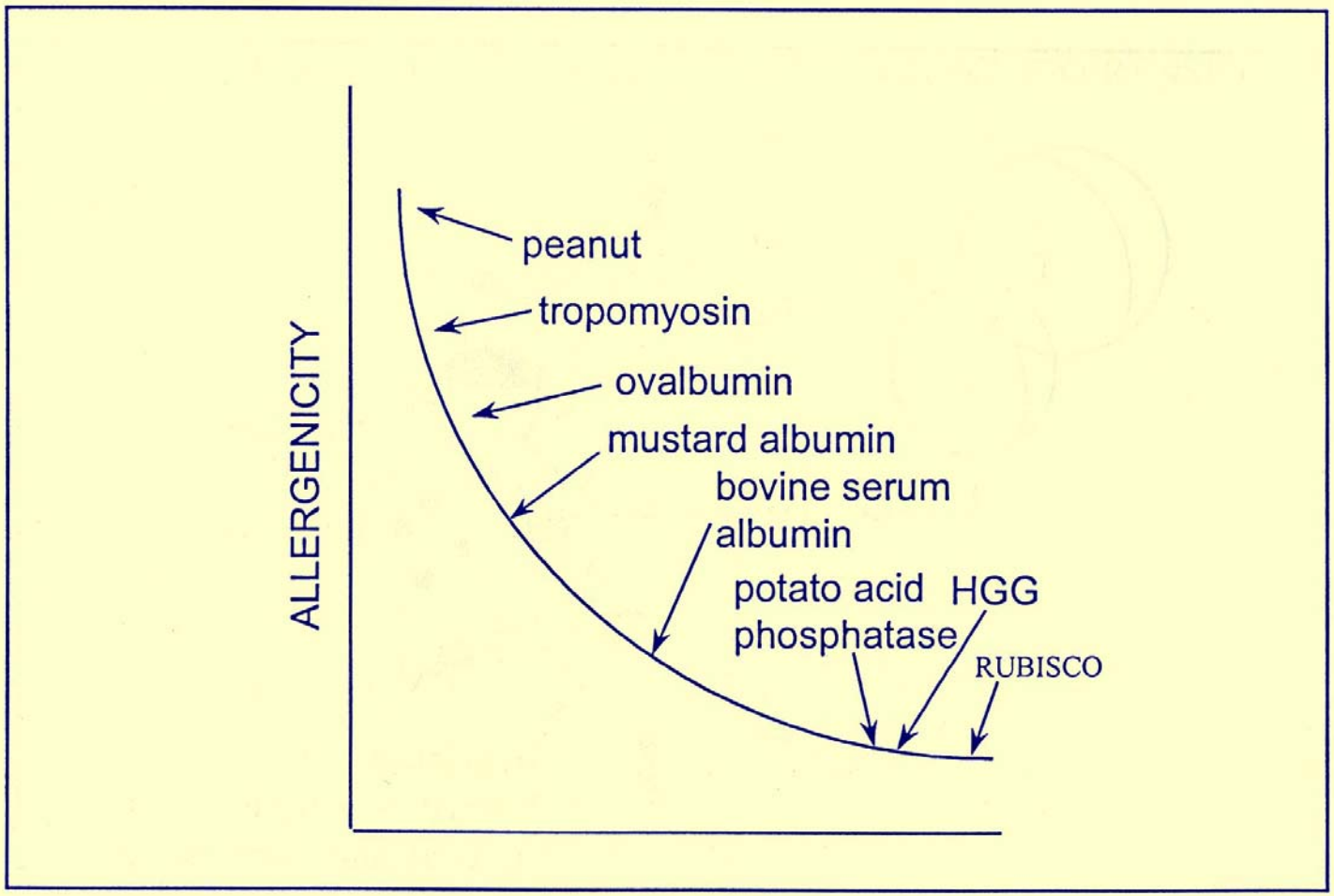
Non-oral routes:

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

Oral route with adjuvant (cholera toxin):

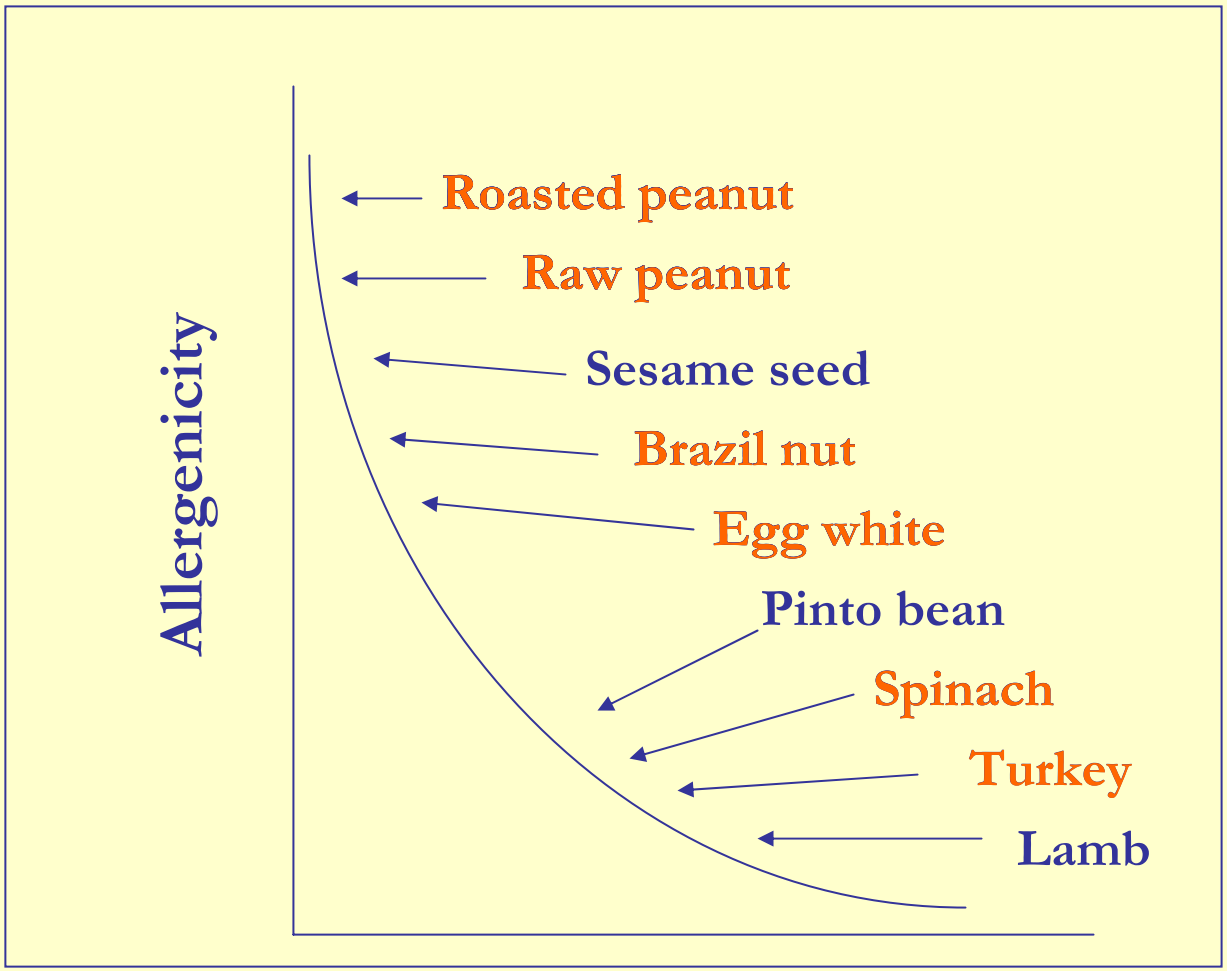
- widely accepted as a model for studying mechanisms of food allergy (peanut, cow's milk, shrimp, etc.)
- normal route of exposure

Spectrum of food allergens



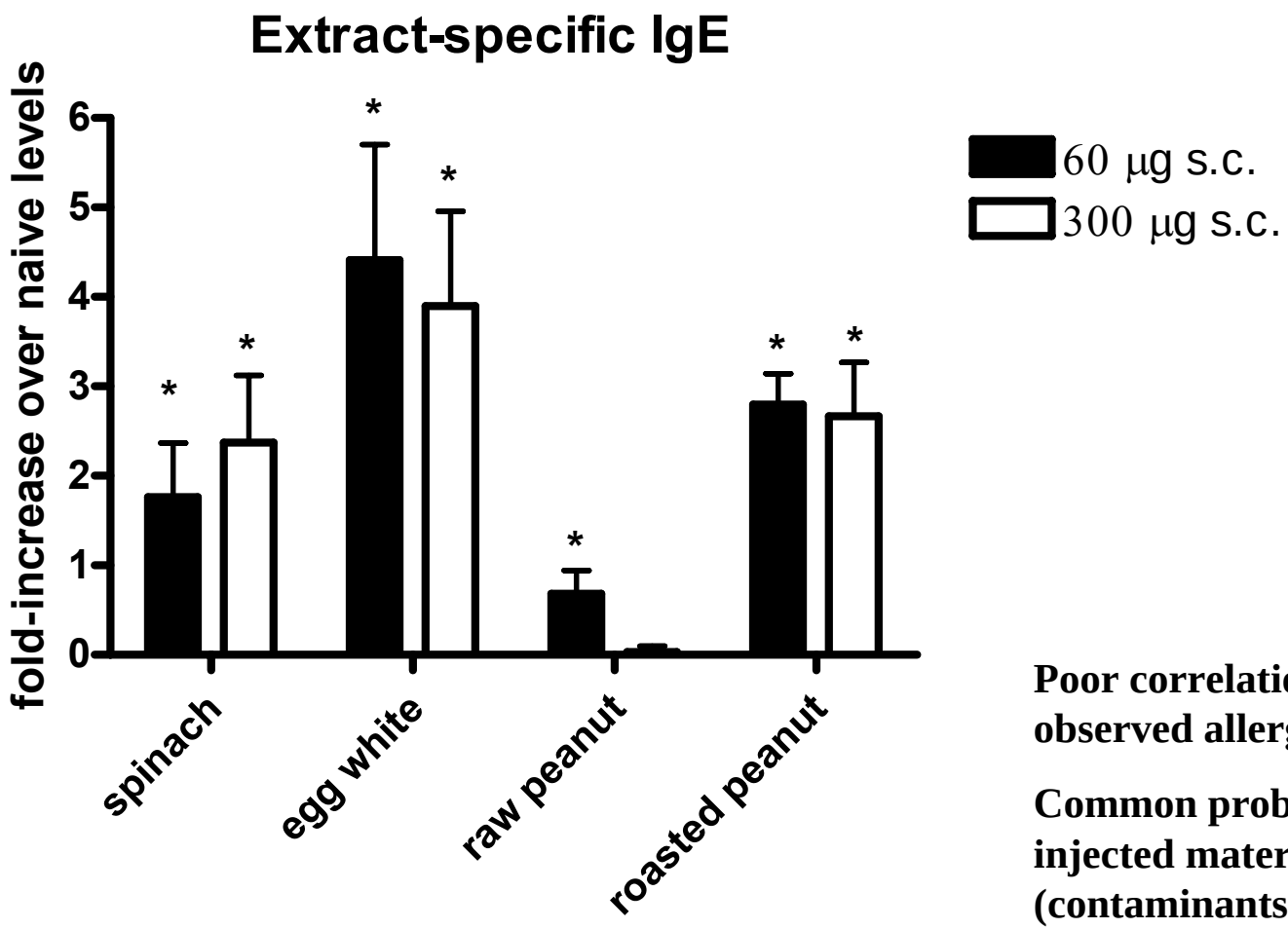
Ian Kimber

Spectrum of test food allergens



Based on observed allergenicity in humans

IgE responses after subcutaneous exposure

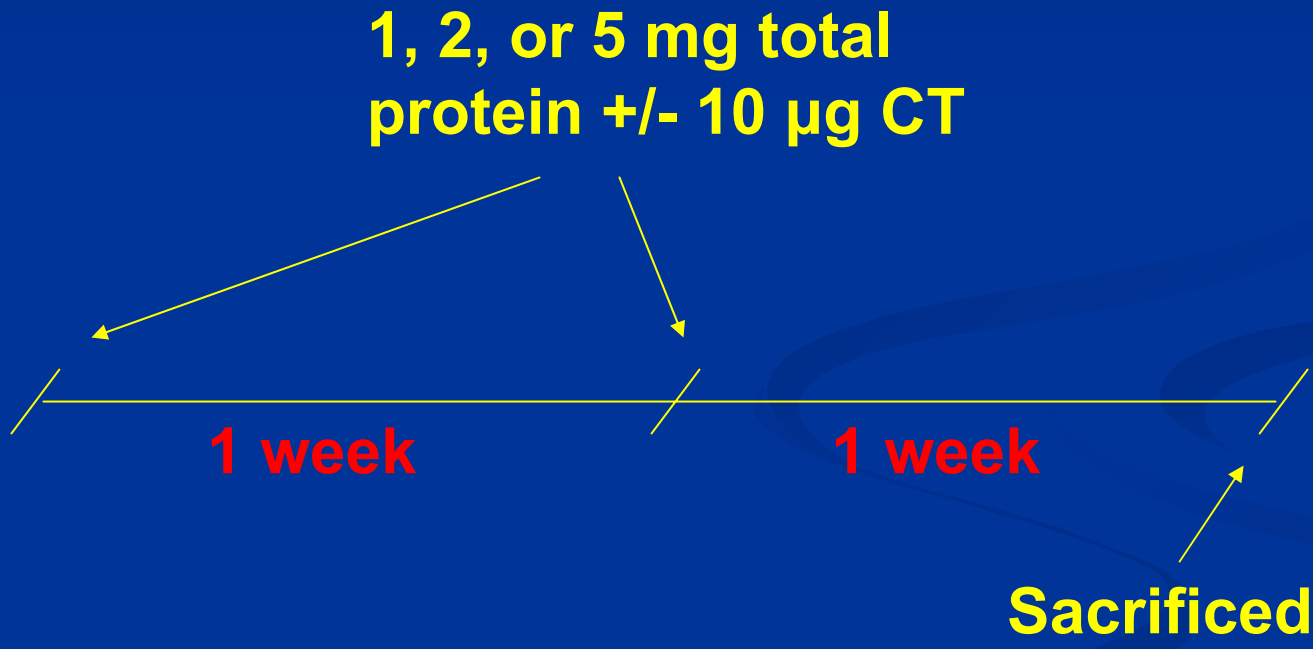


Poor correlation with observed allergenicity

Common problem with injected materials (contaminants)

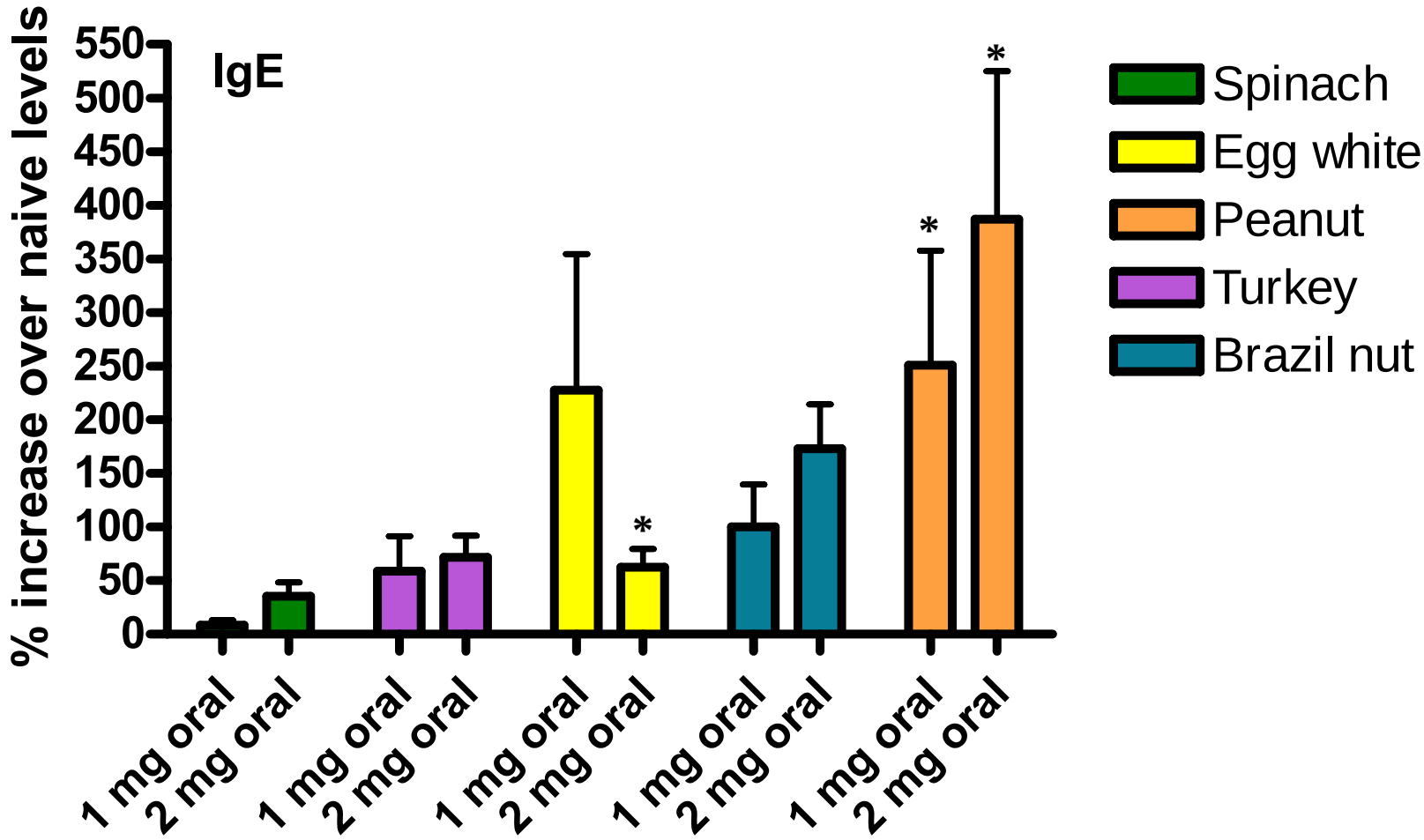
Sensitization of C3H/HeJ mice with food extracts and cholera toxin

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

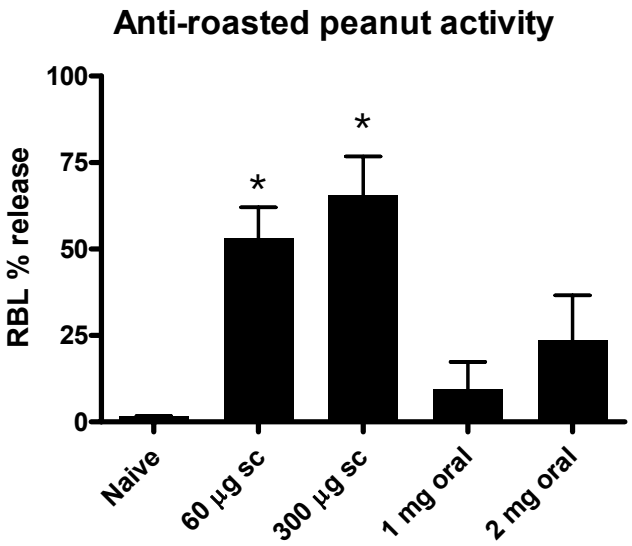


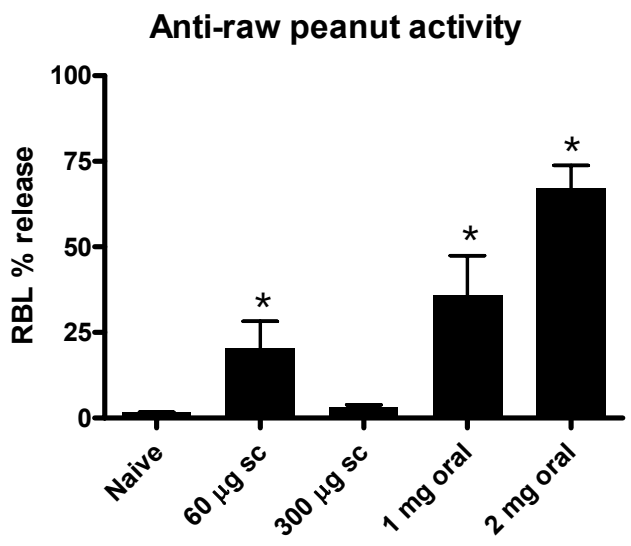
Endpoints: food extract-specific IgE, IgG1, and IgG in serum

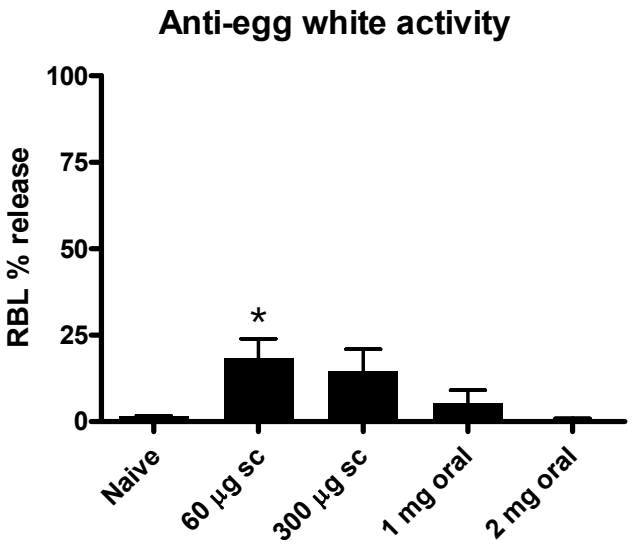
IgE responses after two oral exposures with cholera toxin

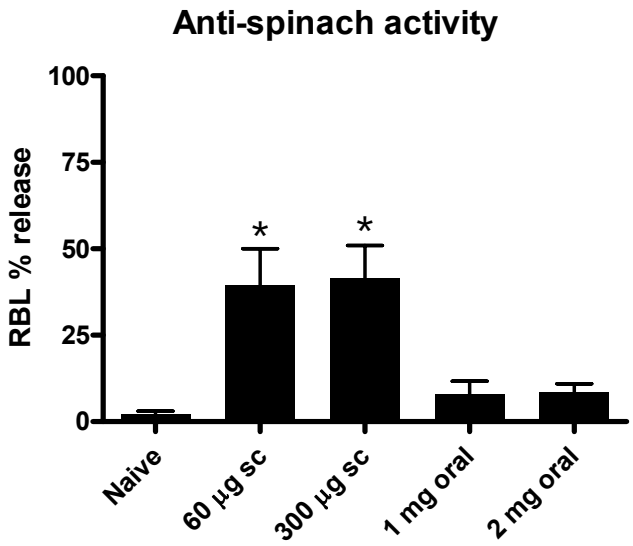


Rat basophil leukemia cell assay for IgE functionality

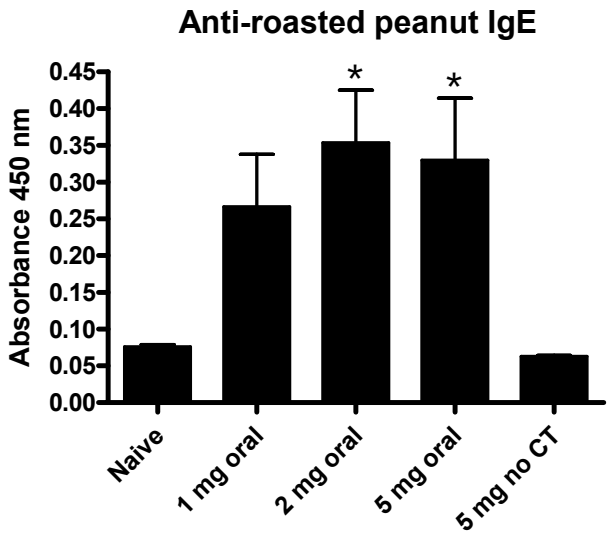


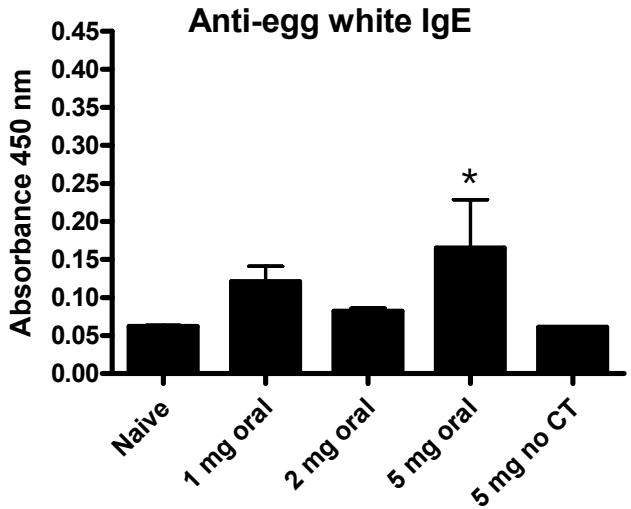


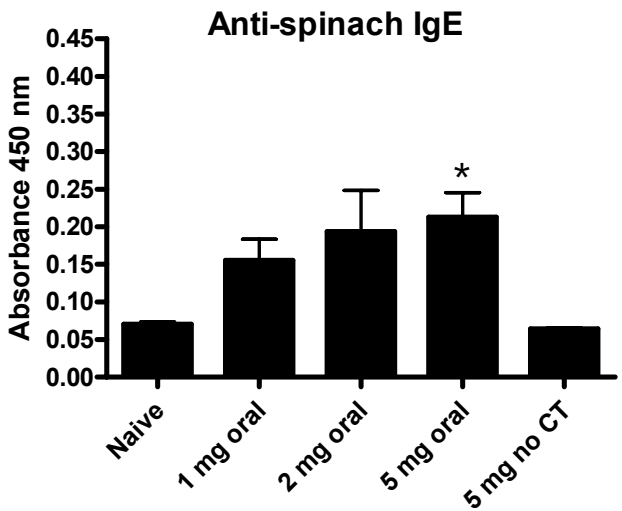




IgE after four oral exposures with CT: loss of selectivity







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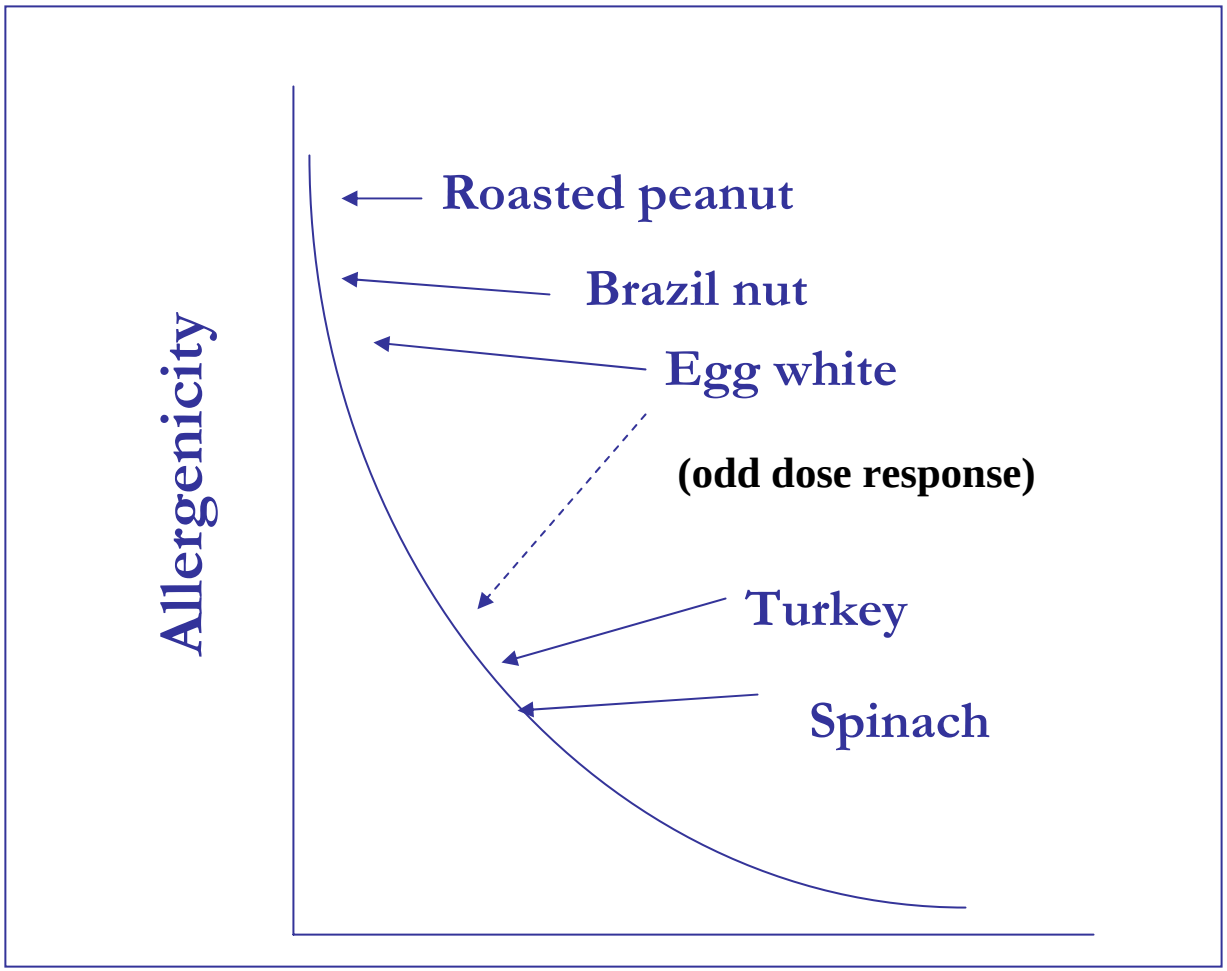
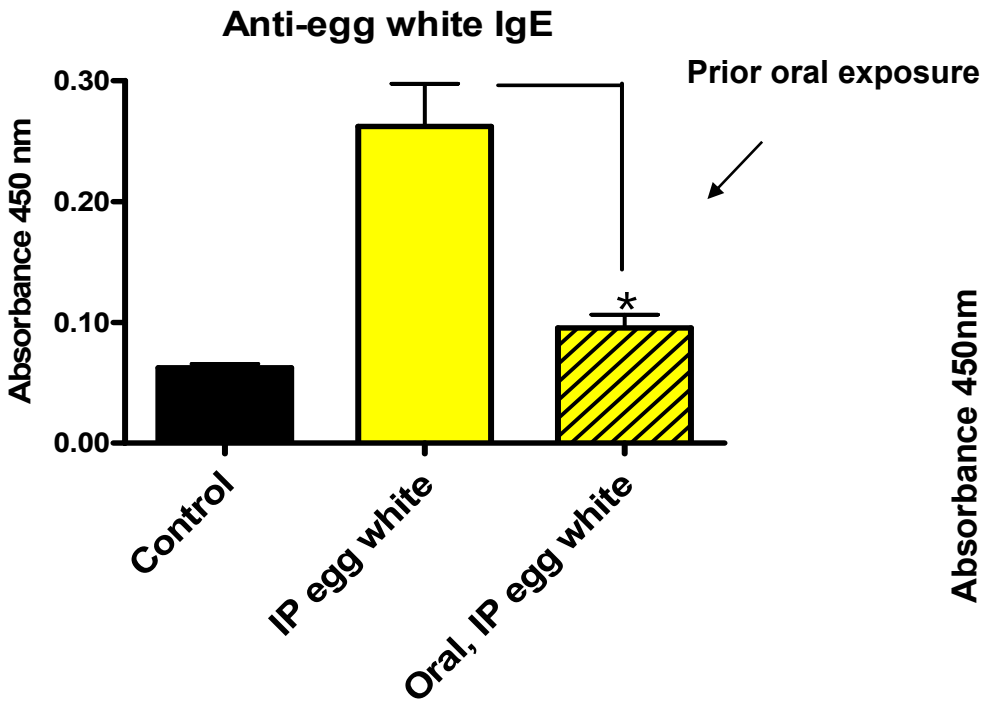


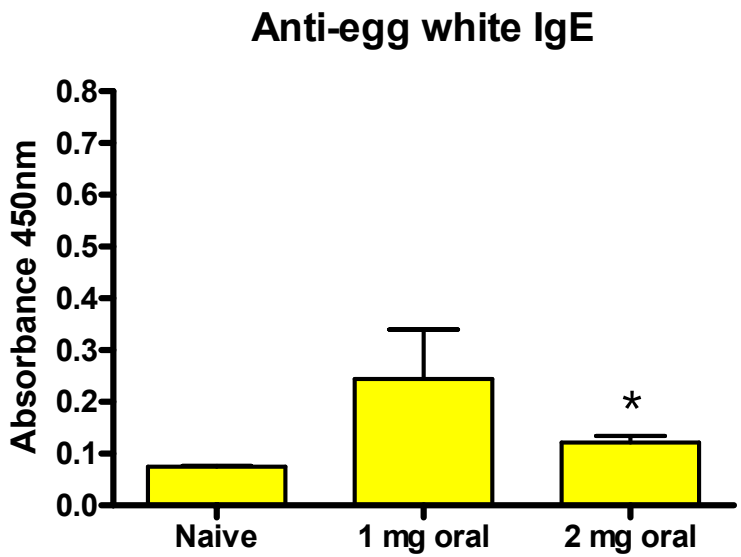
Based on observed allergenicity in mice

Oral tolerance to egg white

vs.

Oral sensitization to egg white

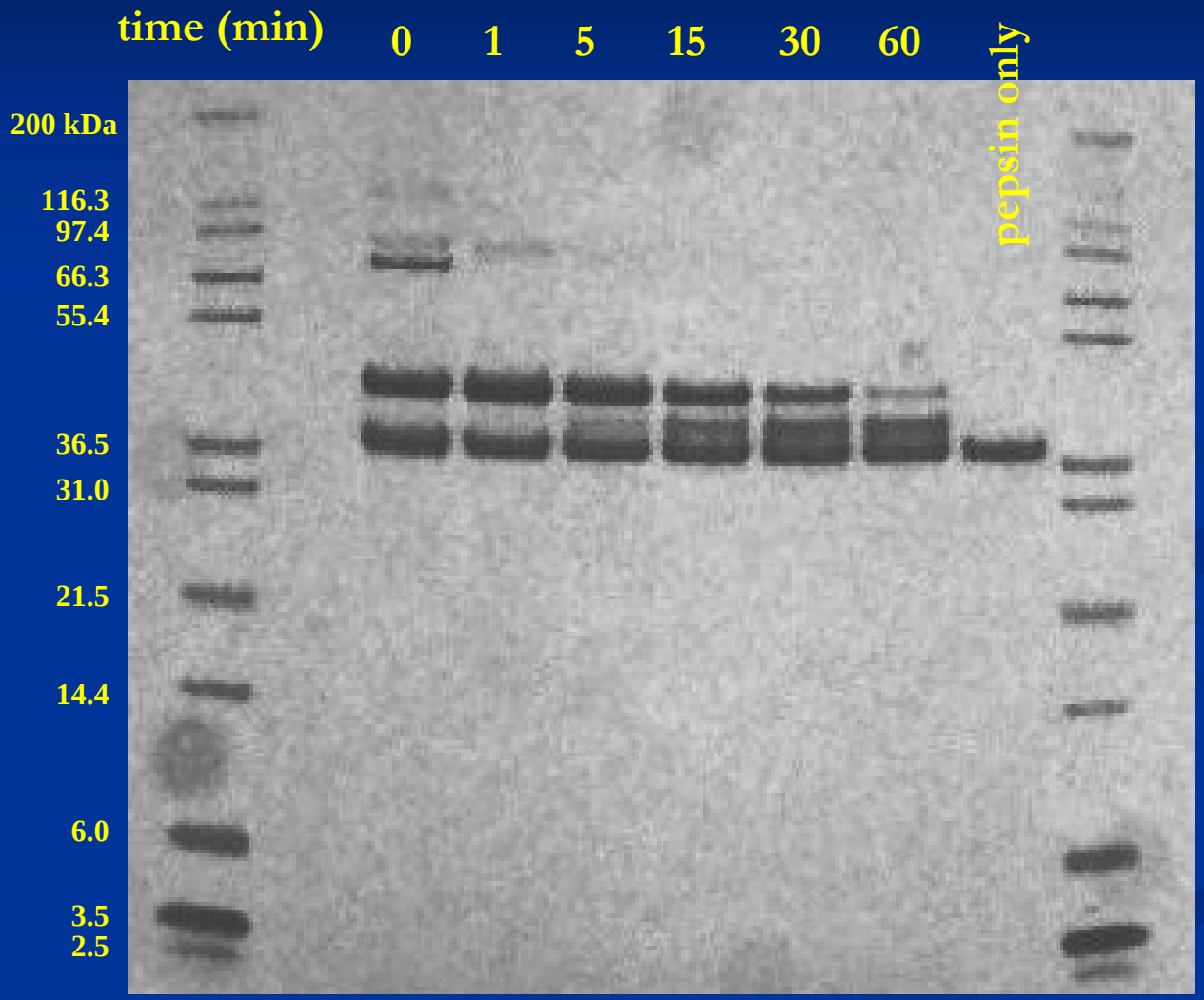




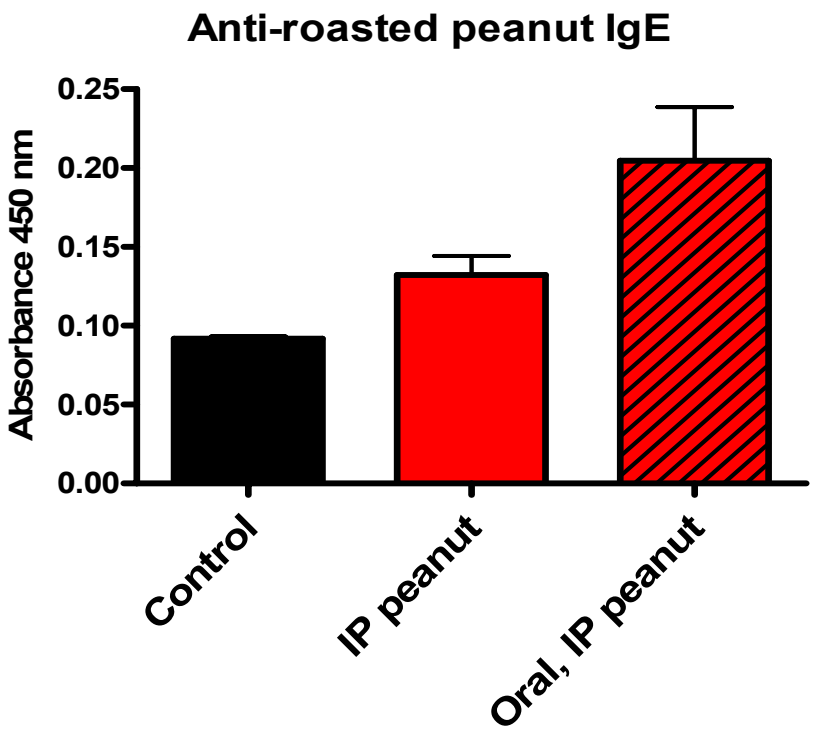
with cholera toxin

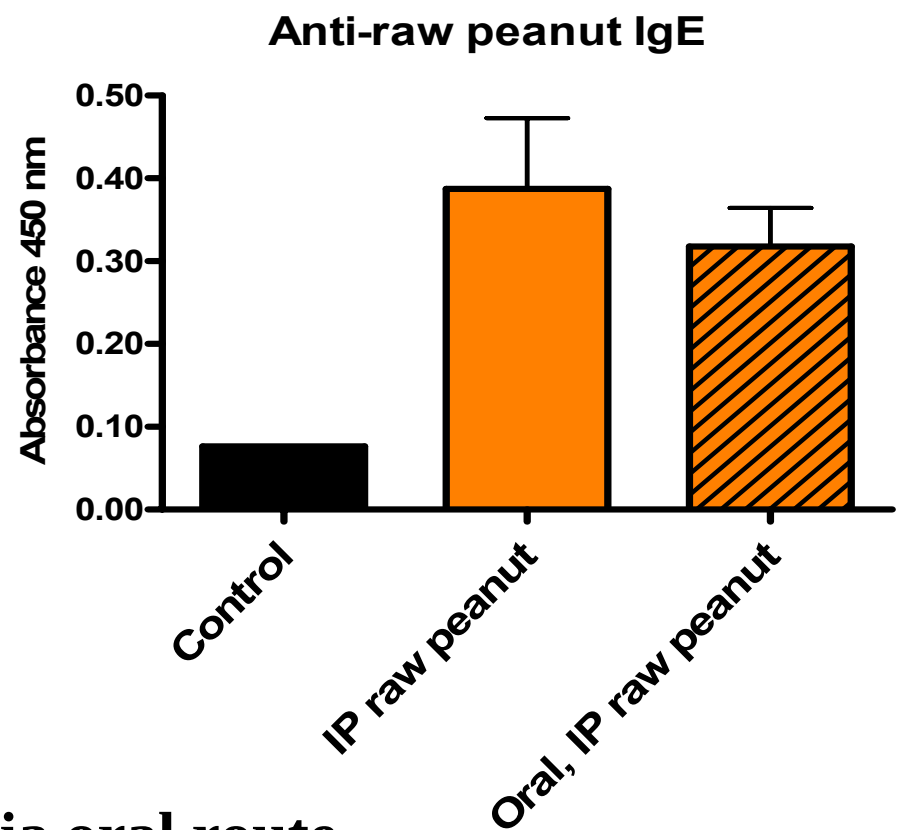
Egg white induces oral tolerance, and has some sensitizing potential but unusual dose responses when administered orally with cholera toxin. Most egg white proteins are readily digested, though stable fragments remain after two hours.

Pepsin digest of egg white



No oral tolerance to peanut



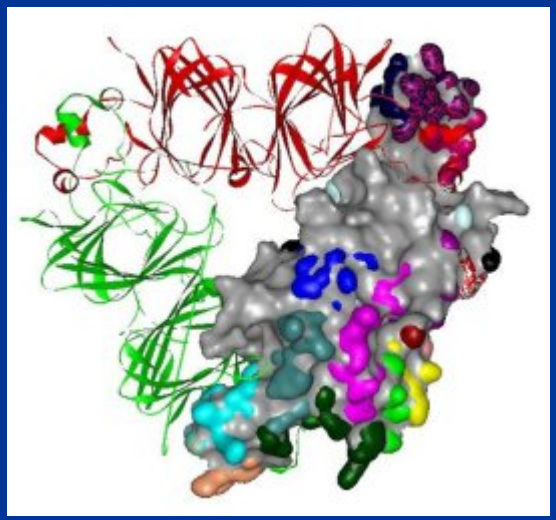


but no IgE without cholera toxin via oral route

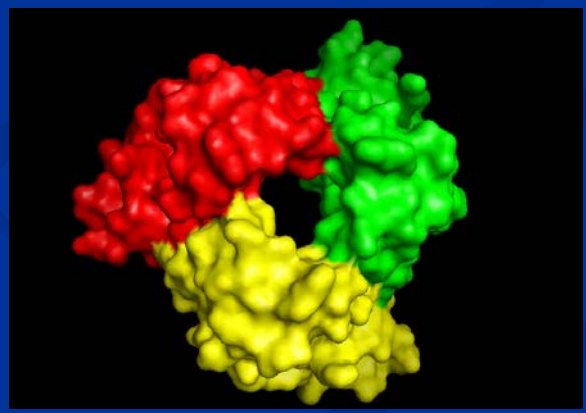
Peanut allergy is only outgrown in 20% of patients

Why are some allergens not subject to oral tolerance?

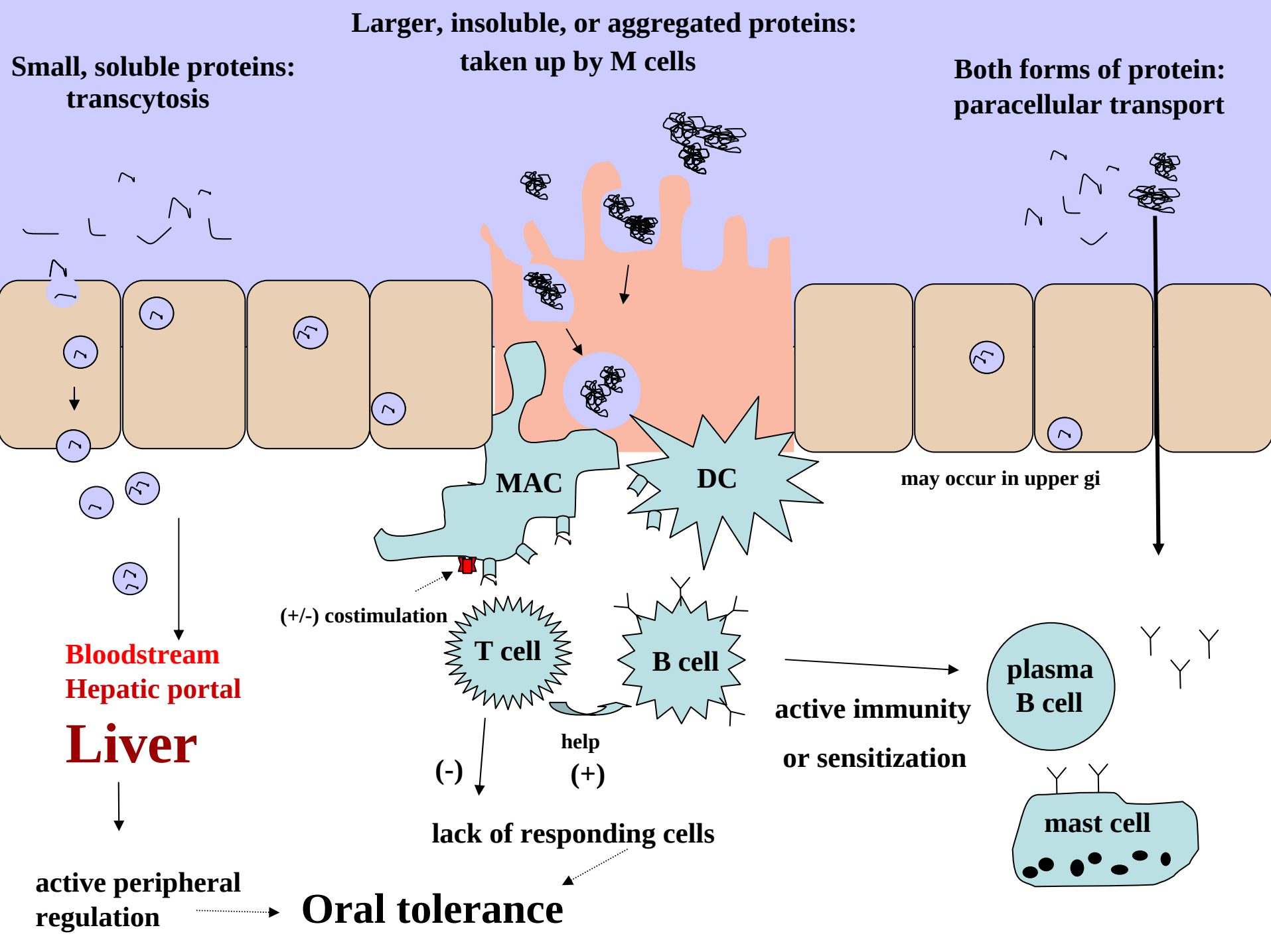
- Digestibility (or solubility) – many allergens resist digestion
- Example: roasted peanut is not very soluble, resists digestion in vitro



Cashew allergen trimer



Peanut allergen trimer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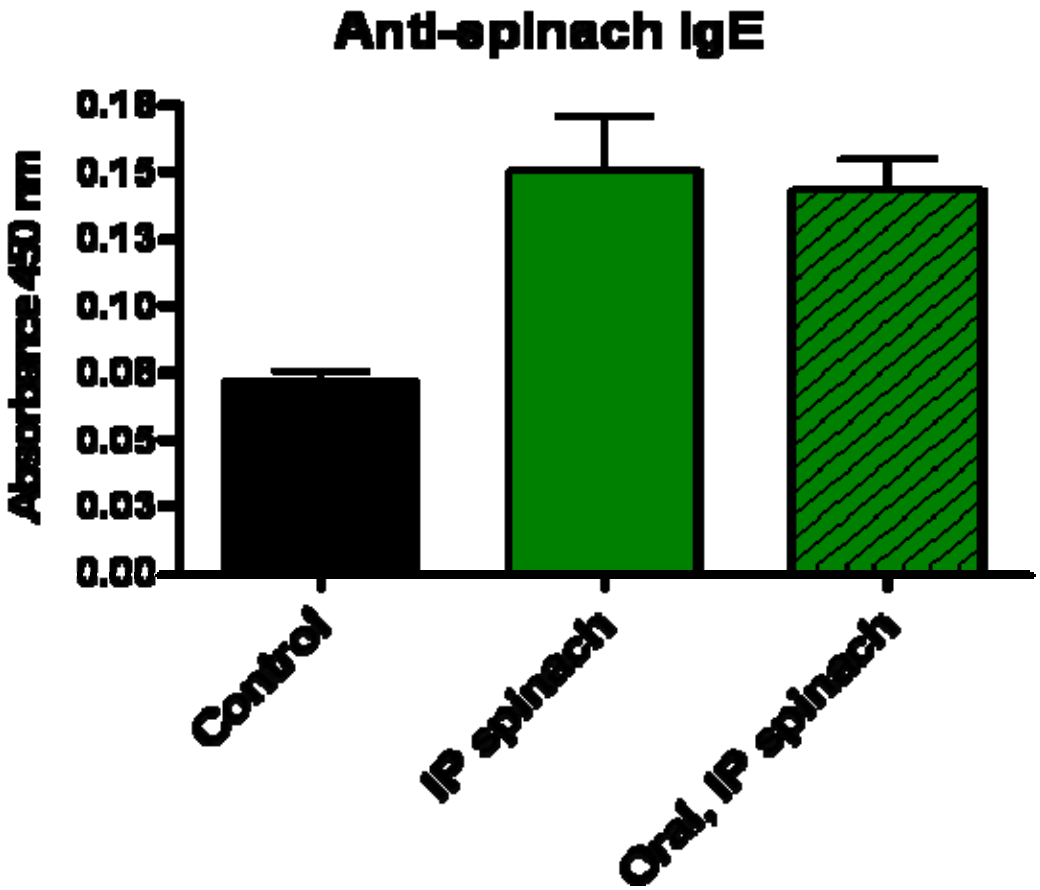
mast cell
lack of responding cells
active peripheral
regulation
Oral tolerance

What about allergens that ARE digestible?

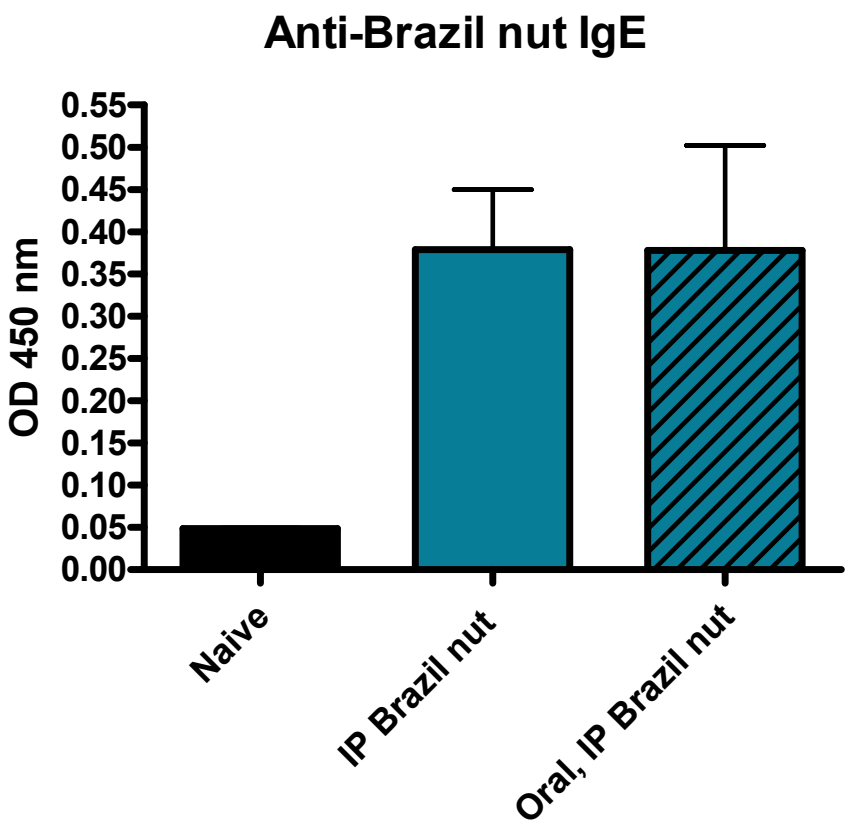
- Frequently cause allergy in children and don't last into adulthood – eggs, milk
- Others cause only oral allergy syndrome in adults (local sensitization, no systemic effects) – fruit & vegetable proteins
- Cross-react with respiratory allergens (pollen); route of sensitization probably not oral!

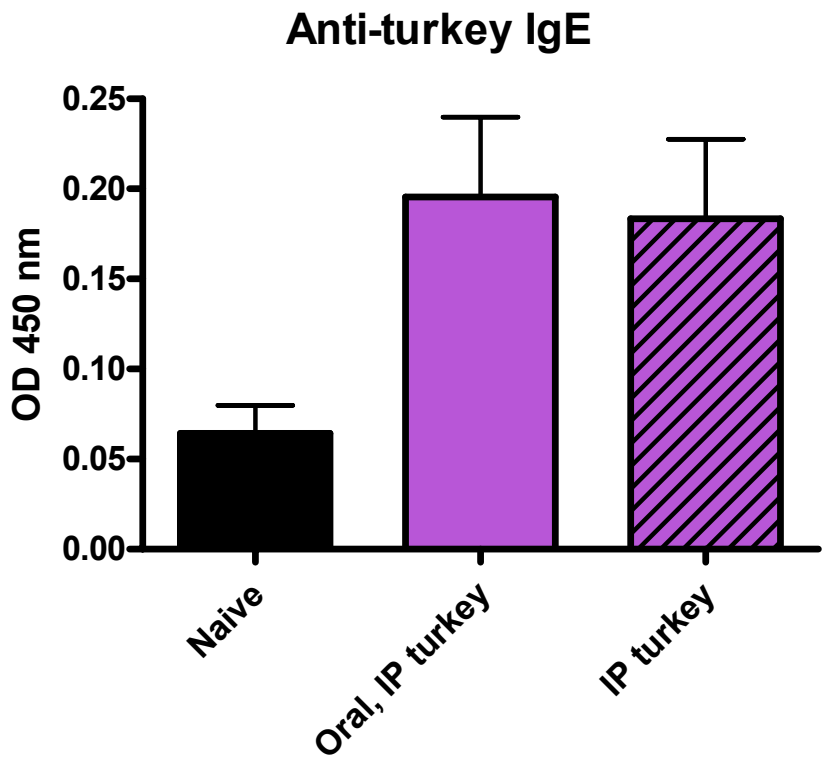
No oral tolerance to spinach

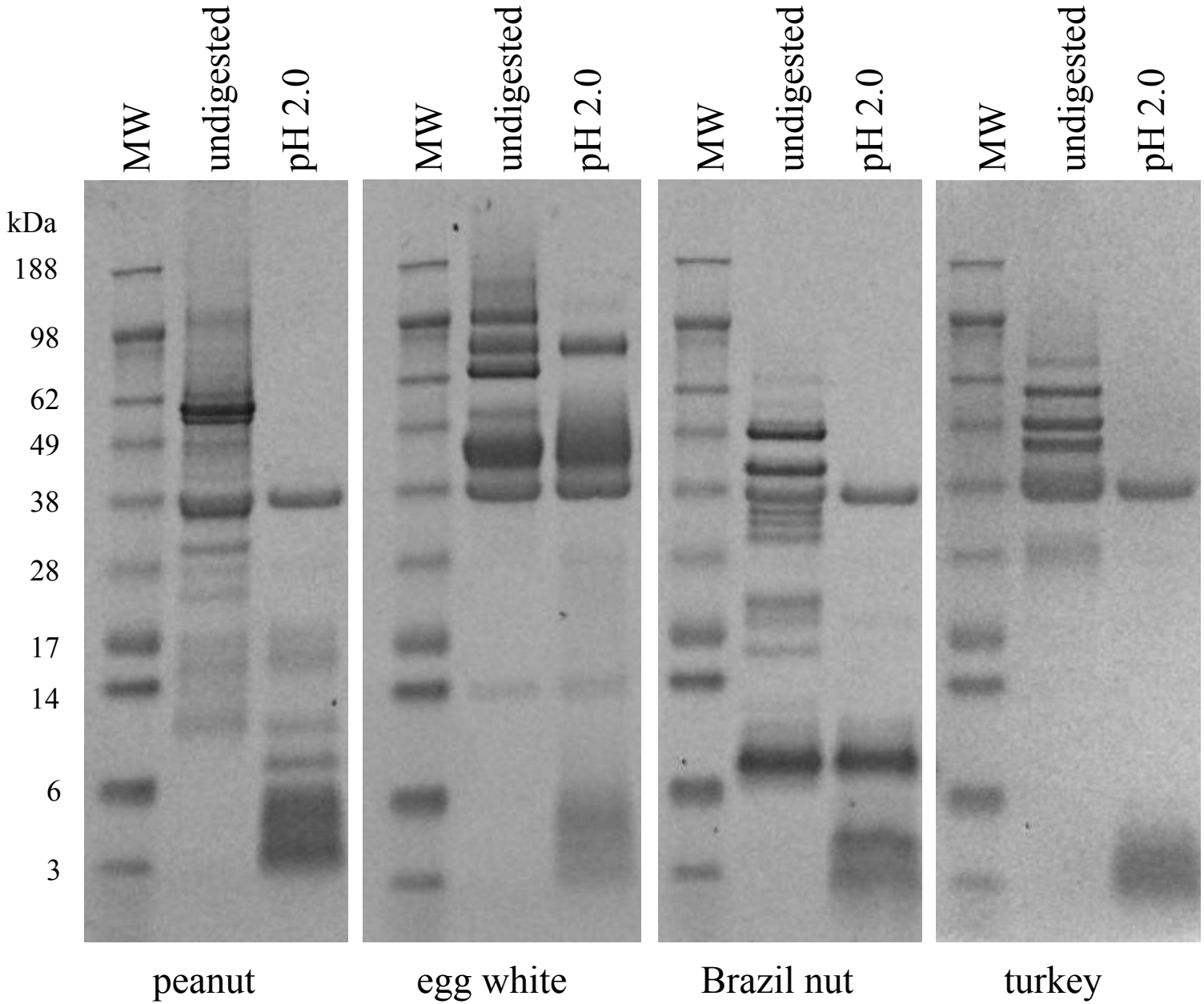


Spinach does not induce oral tolerance, but also has very little sensitizing potential when administered orally with cholera toxin. The major spinach protein is highly digestible with no fragments remaining after 15 seconds.

No oral tolerance to Brazil nut or turkey






MW
undigested
pH 2.0
MW
undigested
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MW
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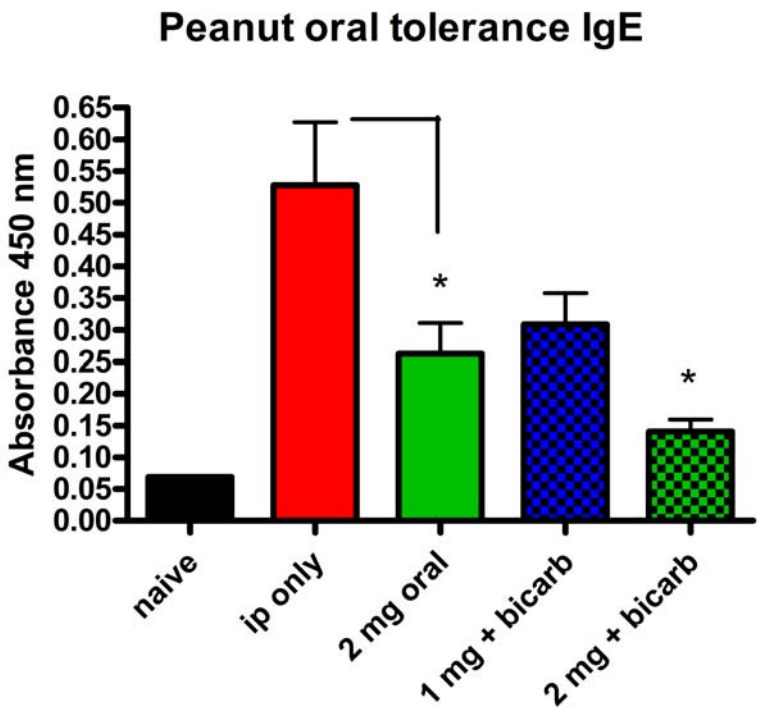
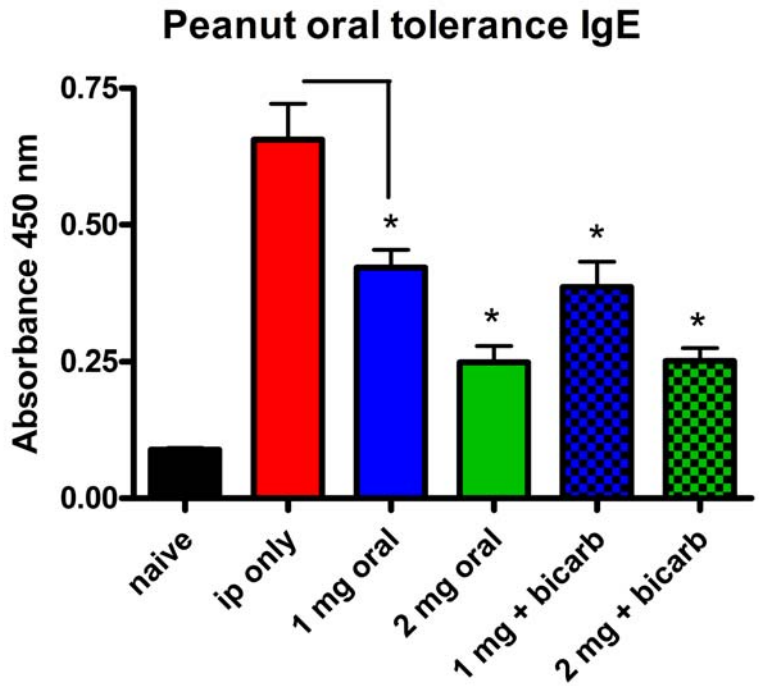
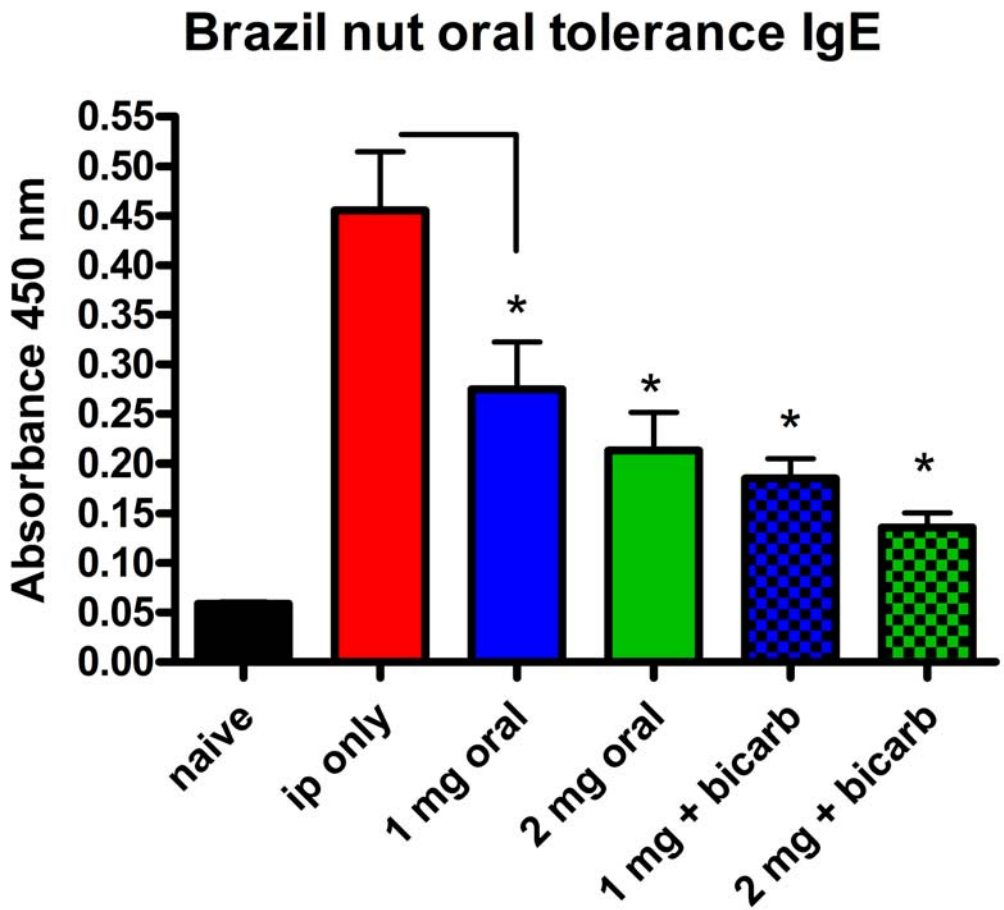
kDa
188
98
62
49
38
28
17
14
6
3
peanut
egg white
Brazil nut
turkey

Manipulation of oral tolerance: peanut





Manipulation of oral tolerance: Brazil nut



Conclusions

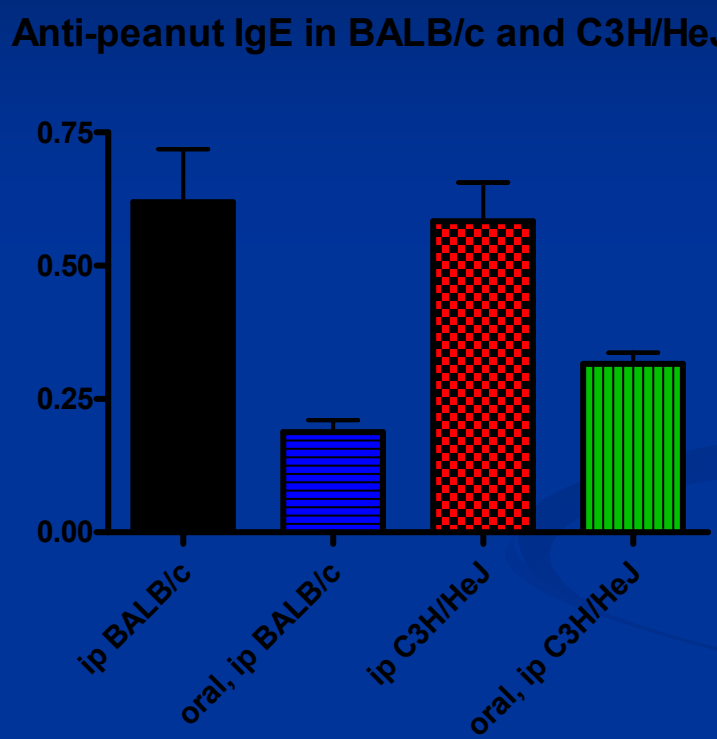
- Digestibility likely plays a role in both the ability to serve as a target for allergic responses and to participate in tolerance when administered orally.
- More thorough analysis of the actual target proteins in each extract is required.
- Additional foods need to be examined in both models and by 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!

Weight of evidence within animal model set

Food	Sensitizing	Tolerizing	
Peanut	+	-	High risk
Brazil nut	+	-	
Egg white	+	+ (*N)	↓
Turkey	-	-	
Spinach	-	-	Low risk

Thank you

- MaryJane 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
0.75
0.50
0.25
0.00
ip BALB/c
oral, ip BALB/c
ip C3H/HeJ
oral, ip C3H/HeJ

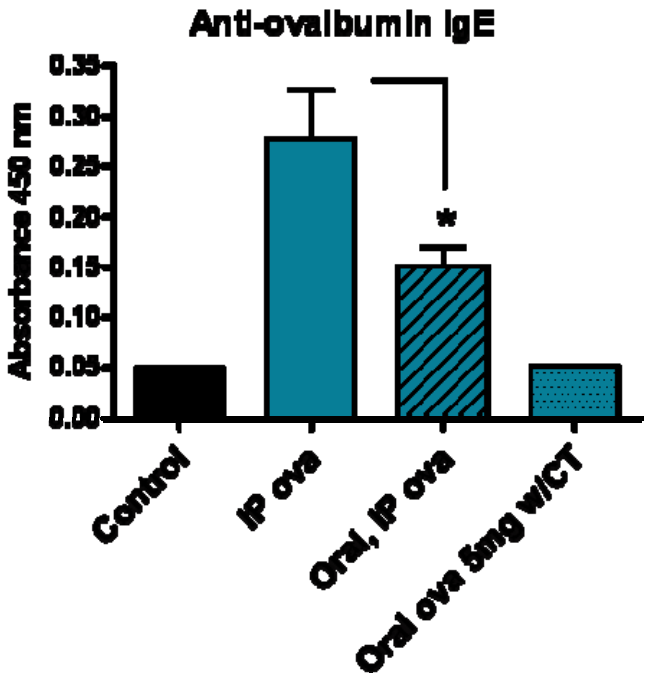
Stability of individual food proteins in simulated gastric fluid

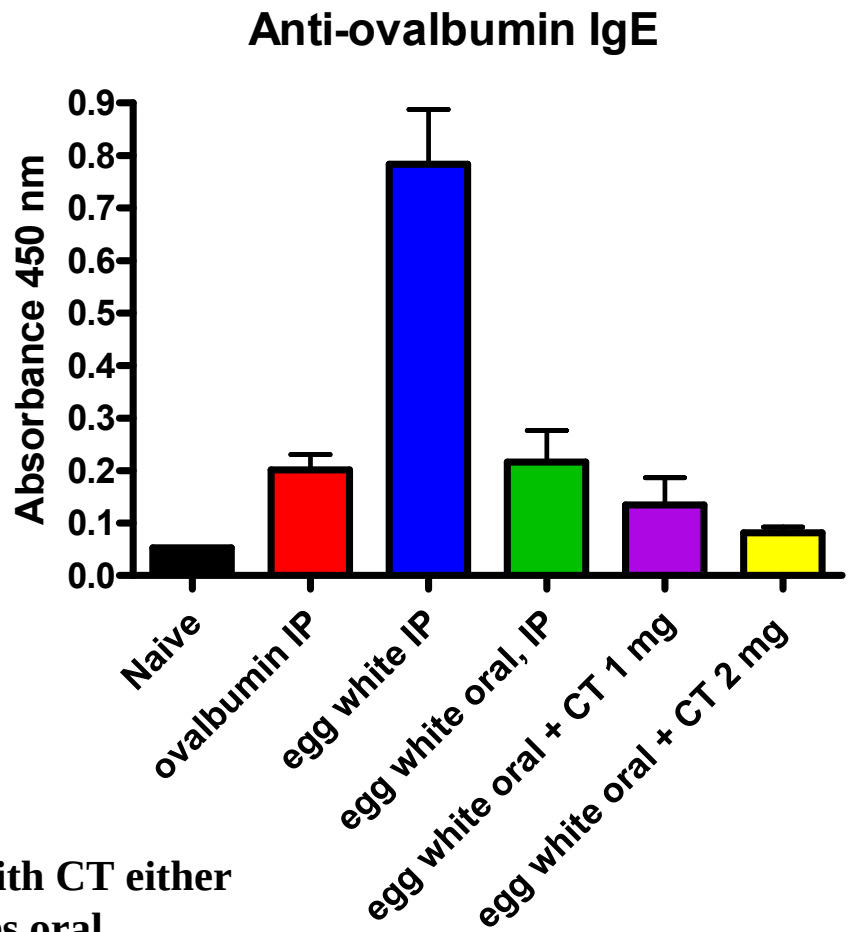
Results from several studies are reported as minutes to digestion with persistence of remaining fragments in parenthesis.

Food	Protein	Study #1	Study #2	Study #3	Relative stability
Peanut	Ara h1	nda	5	0 (120)	-
	Ara h2	60	15 (120)	120 (120)	+ +
Egg white	Ovalbumin	60	5	60 (120)	+
	Ovomucoid	8	0	0 (5)	-
Spinach	Rubisco	0	0	nda	- -

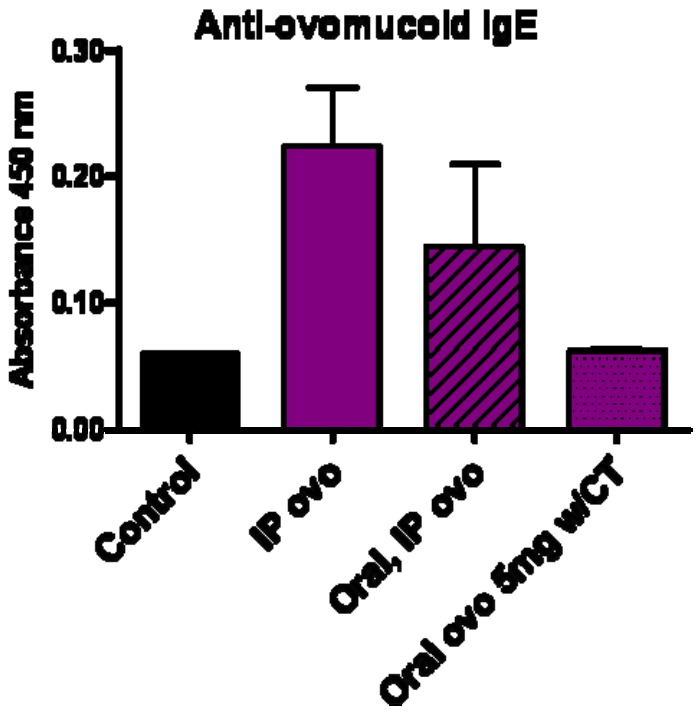
Study #1: Astwood, J.D., et al., 1996[1]
Study #2: Fu, T.J., et al., 2002[2]; enzyme to substrate ratio 10/1
Study #3: Fu, T.J., 2006 (personal communication); enzyme to substrate ratio 1/1
nda = no data available

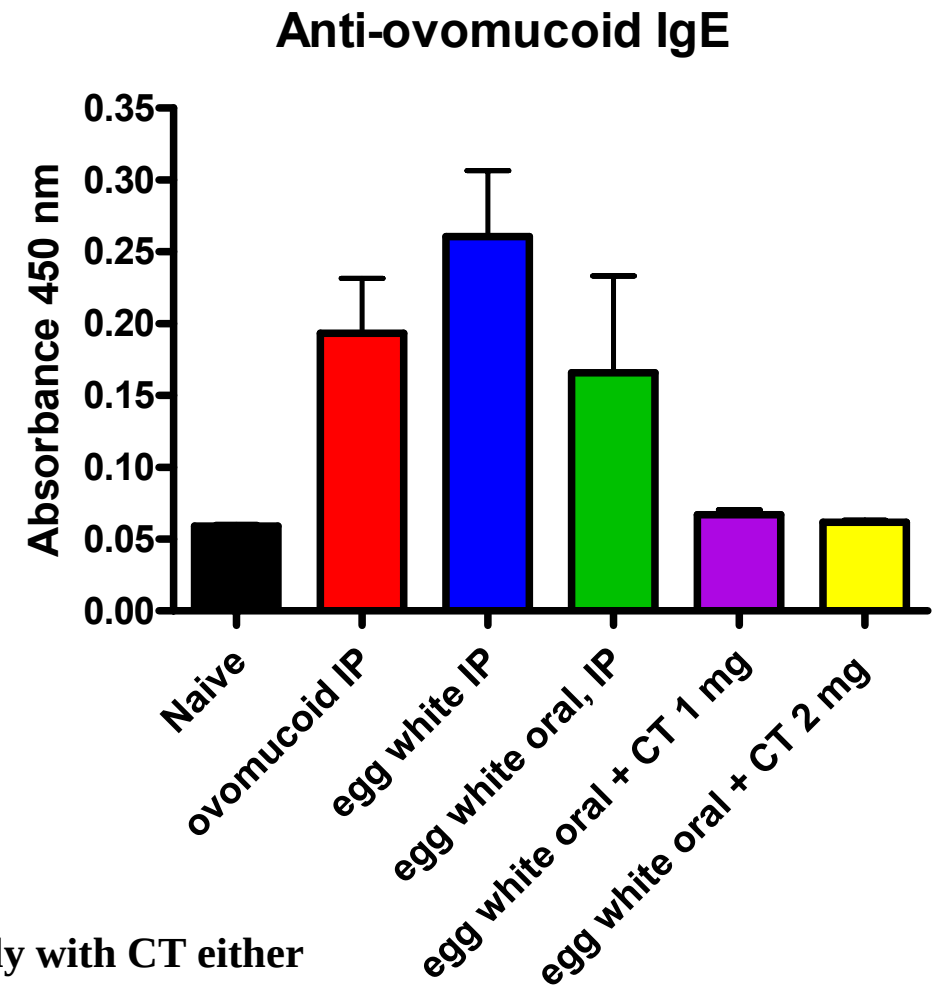
Egg protein-specific responses



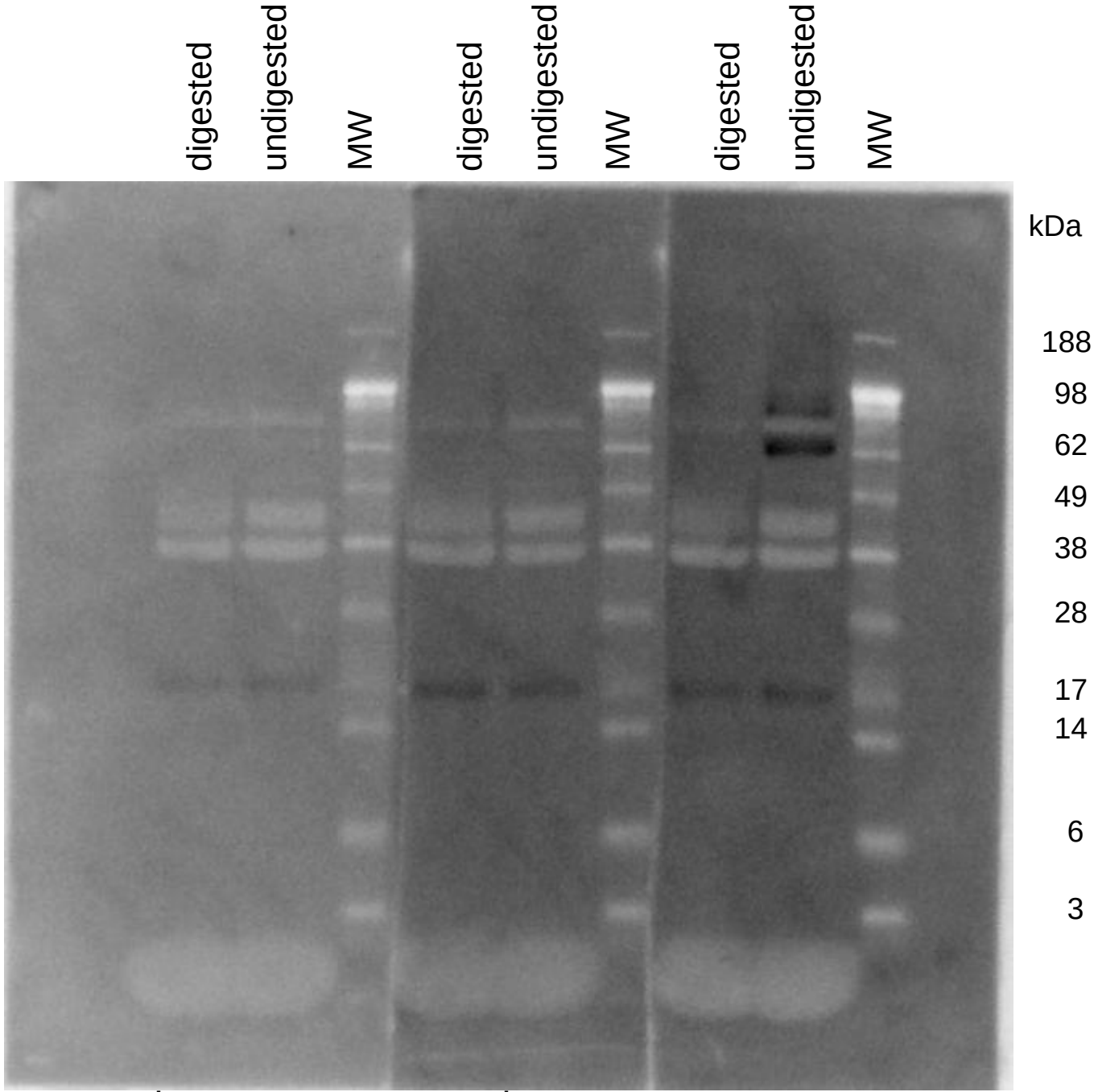


Ovalbumin is not sensitizing when given orally with CT either alone or as a component of egg white. Ova induces oral tolerance.



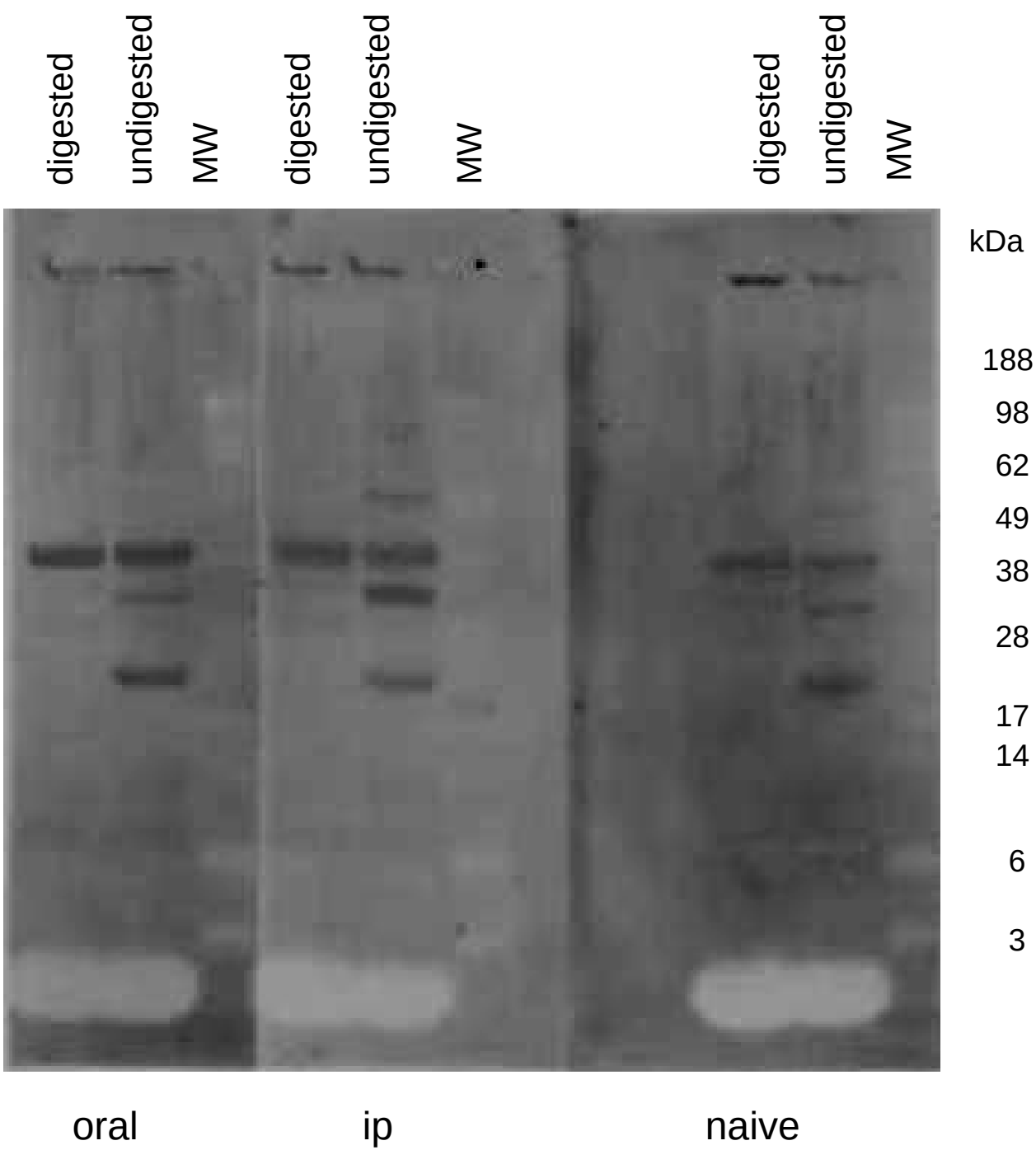


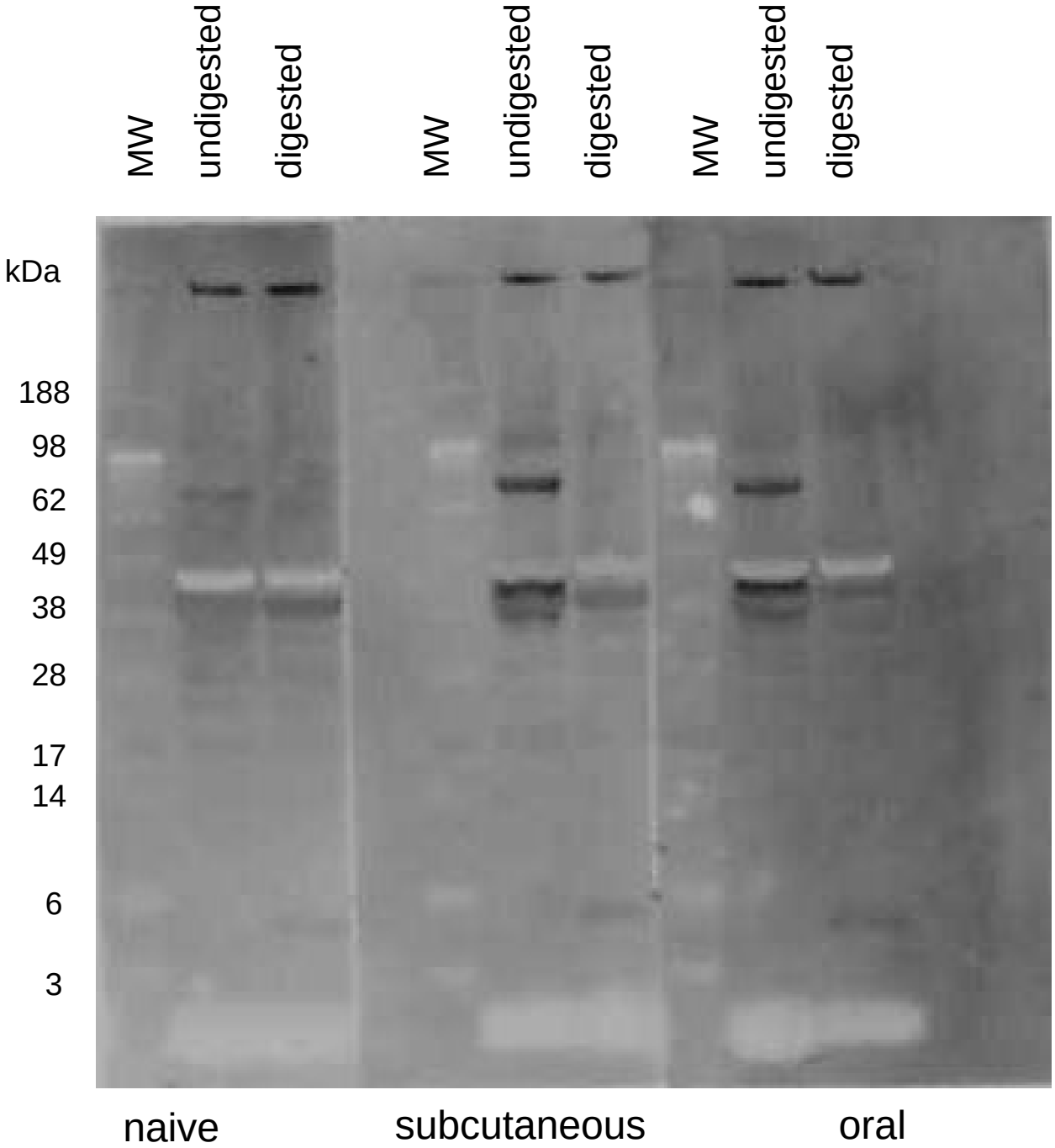
Ovomucoid is not sensitizing when given orally with CT either alone or as a component of egg white.



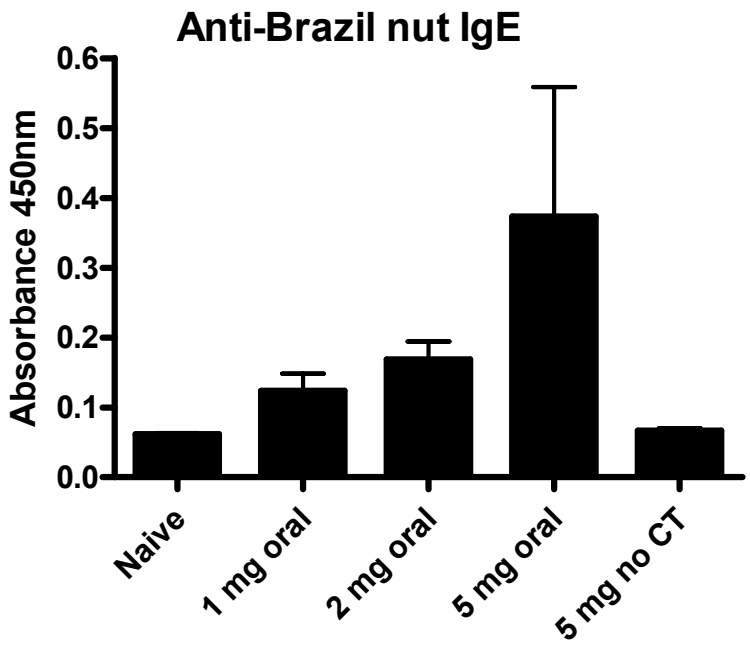
egg white
western
7-31-07

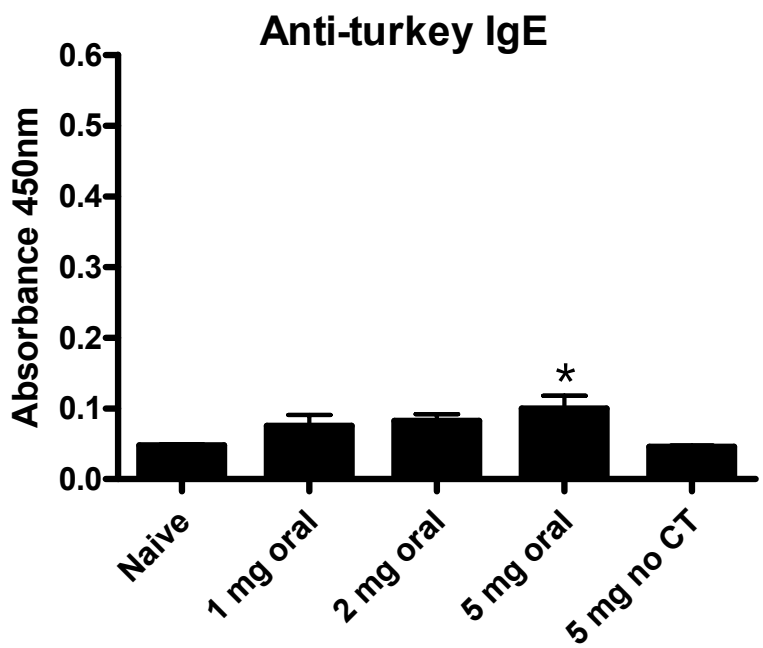
brazil nut
western
8-3-07




MW
undigested
digested
MW
undigested
digested
MW
undigested
digested
kDa
188
98
62
49
38
28
17
14
6
3
naive
subcutaneous
oral
oral sera
from 4x imm
egg white
western
8-3-07

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