The rat Brown Norway model to asses the oral sensitizing properties of food proteins

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Special acknowledgement

Léon Knippels, PhD

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Wageningen
The Netherlands

and colleagues involved in the studies conducted in the BN rat model.

Why Should We Use Animal Models?

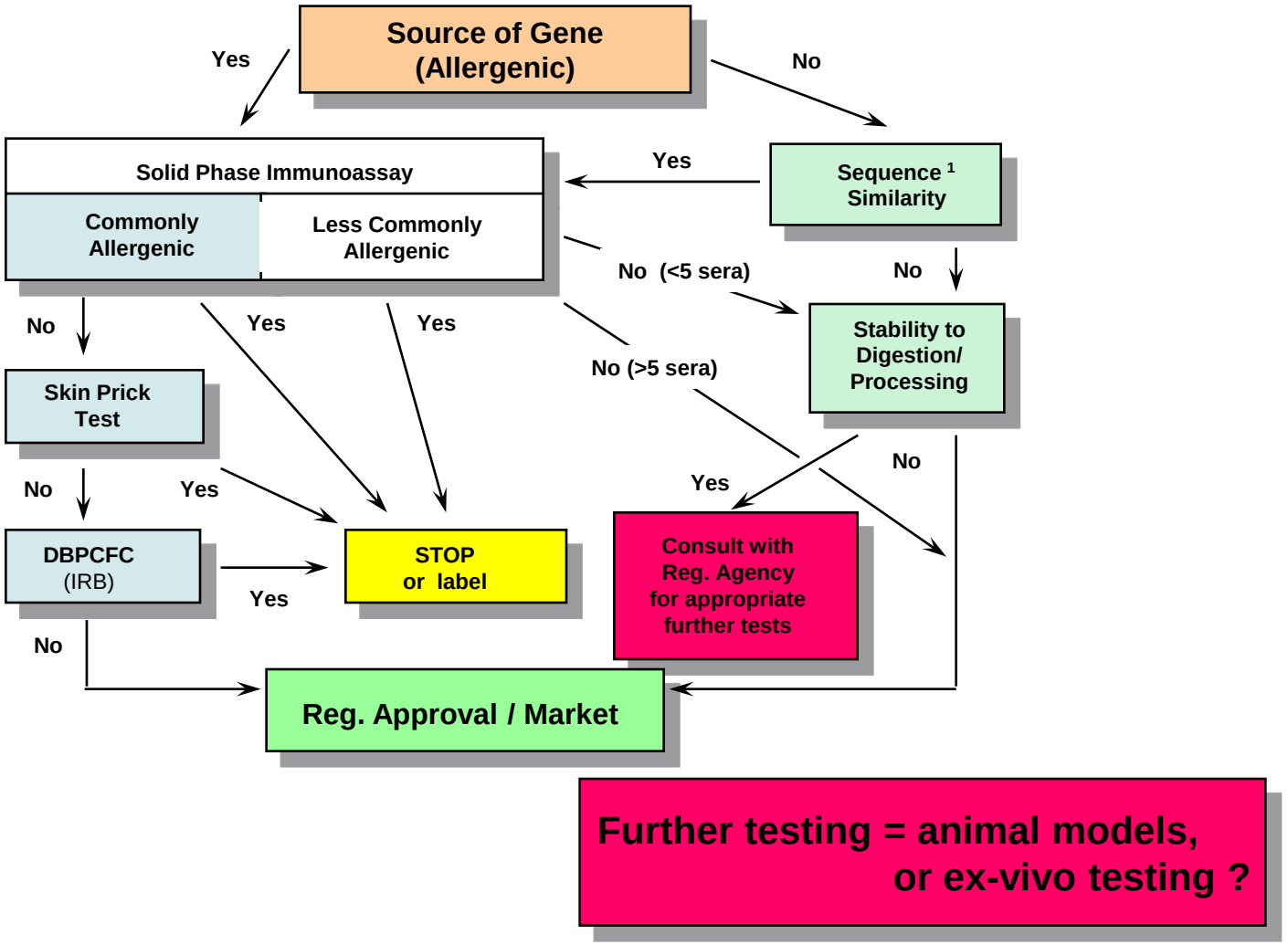
- Sensitization studies in humans not possible, only challenge reaction can be studied in patients
- Mechanistic research of IgE-mediated food allergy
- Development of methods for prevention or therapy of food allergy
- Prediction of potential allergenicity of new food proteins (e.g. GMO's), ranking of the relative allergenicity

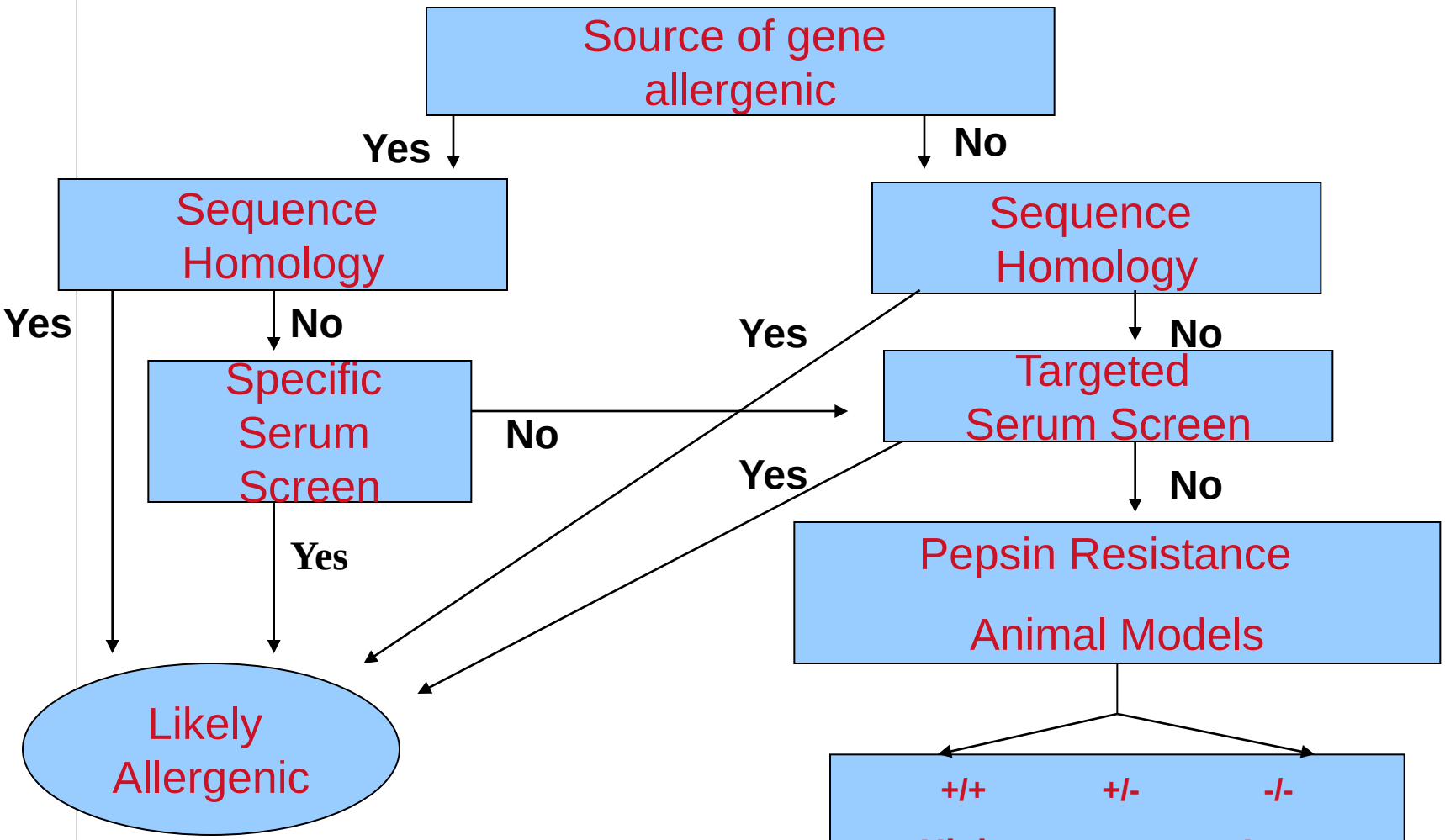
Prediction of Food Allergy

More attention for animal models predicting potential allergenicity was raised following the introduction of GMO's:

- Is a protein that was not previously common in the human diet likely to be allergenic if introduced in commodity crops?
- Will production of biotechnology (GM) crops alter the allergenicity of endogenous proteins?

IFBC Allergenicity Assessment for GM crops





FAO/WHO 2001 Decission Tree

Key factors for animal models (1)

- Simple protocols for sensitization and challenges which are reproducible between laboratories and over time, and which take into account:

- Genetic predisposition (species/strain-high/low responder/atopic prevalence)

- Unscheduled dietary pre-exposure of test animals to test protein (diet, parents)

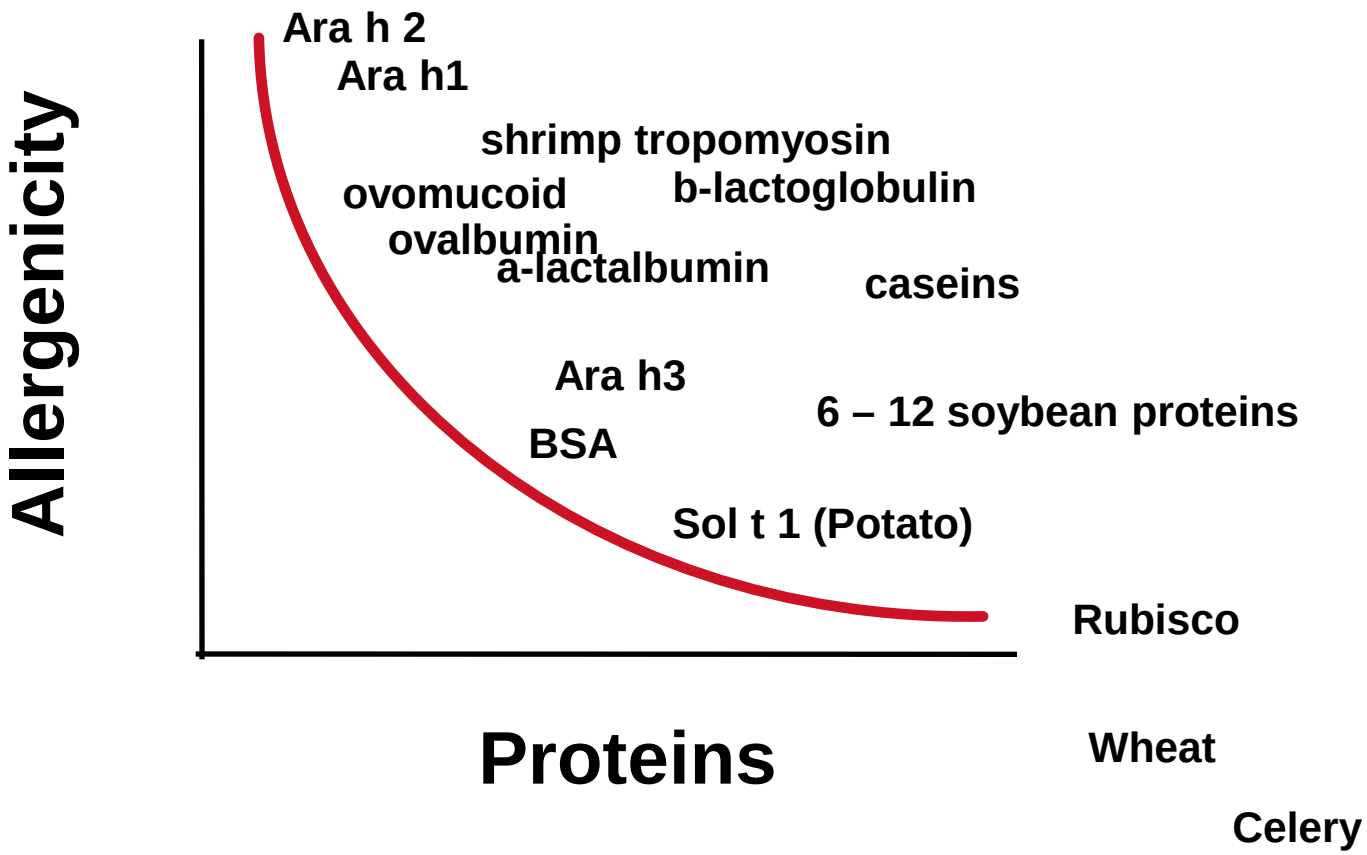
- Sensitization

✓age – neonate, adolescent, adult
✓route – oral/gavage/i.p./dermal/subcutaneous.
✓test material – whole foods/purified proteins
✓dose frequency- daily, twice weekly, weekly etc
✓Dose amount – high dose (tolerance)/low dose (sensitization)
✓Use of adjuvant [no adjuvant, cholera toxin (oral), Alum (i.p.)]

Key factors for animal models(2)

- Tolerant to most food proteins
- Comparable allergenicity – strong/week/non-allergenic
- IgE response to comparable proteins as found in patients
- Clinical reactions upon challenge

Relative Food Allergenicity
- Animal Models (severity) -



Animal Models

- Predictive models
  - Brown Norway rat - oral/i.p. (Knippels et al, from 1998)
  - Balb/c mice - i.p., no adjuvant (Dearman et al, from 2000)
  - Balb/c mice - oral, CT (Adel-Patient et al, 2005)
  - B10A mice - i.p./nasal (Akiyama et al, 2001)
  - Guinea pig - oral/gavage/drinking water/i.p. (Kitagawa et al, 1995)
  - Dog - food with adjuvant (Ermel et al, 1997)

- Mechanistic models
  - C3H/HeJ mice - oral with CT (Li et al, from 1999, 2000; van Wijk et al, from 2004)
  - DBA/2 mice (Ito et al, 1997)
  - Swine (Helm et al, 2002, 2003)

Each model has pro's and con's and potential problems

Brown Norway rat food allergy model

- **Several dosing protocols studied in BN rats:**
  - **Ad libitum via drinking water; 0.002, 0.02, 0.2, 2 and 20 mg/mL OVA for 6 weeks**
  - **Gavage dosing of 1 mg/kg OVA, daily, once a week, twice a week, once every two week**

Knippels et al, Clin Exp Allergy, 1998, 28, 368-375.

- **Several rat strains studied with OVA 1mg/rat for 6 wks:**
  - **BN rats**
  - **Hooded Lister (HL)**
  - **Piebold Viral Glaxo (PVG)**
  - **Wistar**

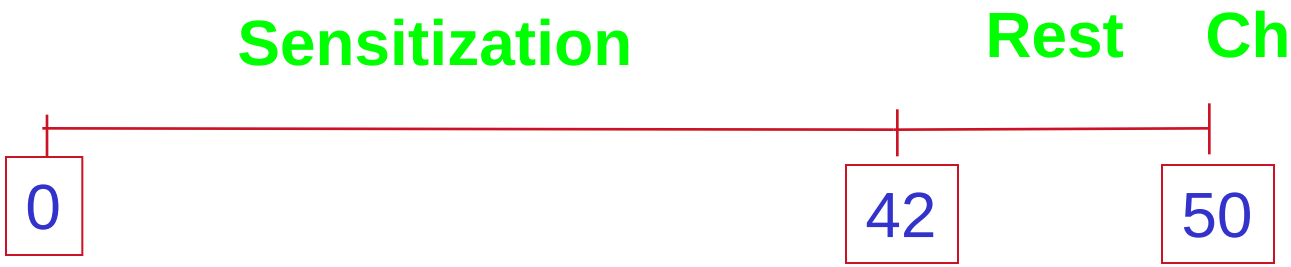
IgG in all strains from d 7, IgE only in BN rats

Knippels et al, Fd Chem Toxicol, 1999, 37, 881-888.

Brown Norway rat food allergy model

- Young male Brown Norway rats (4-6 wks), bred and raised on a test protein free diet (e.g. OVA, egg white, cow's milk free diets).
- 1 mg protein/ml/rat/day by gavage dosing for 42 days
- no adjuvants
- Specific IgG1, IgG2a and IgE responses
- Specific T cell proliferation
- Clinical symptoms after oral challenge
- Comparison of antibody responses to allergic food proteins in BN rats to those in allergic patients

Sensitization and challenge in the BN rat allergy model



Daily gavage 1 mg protein

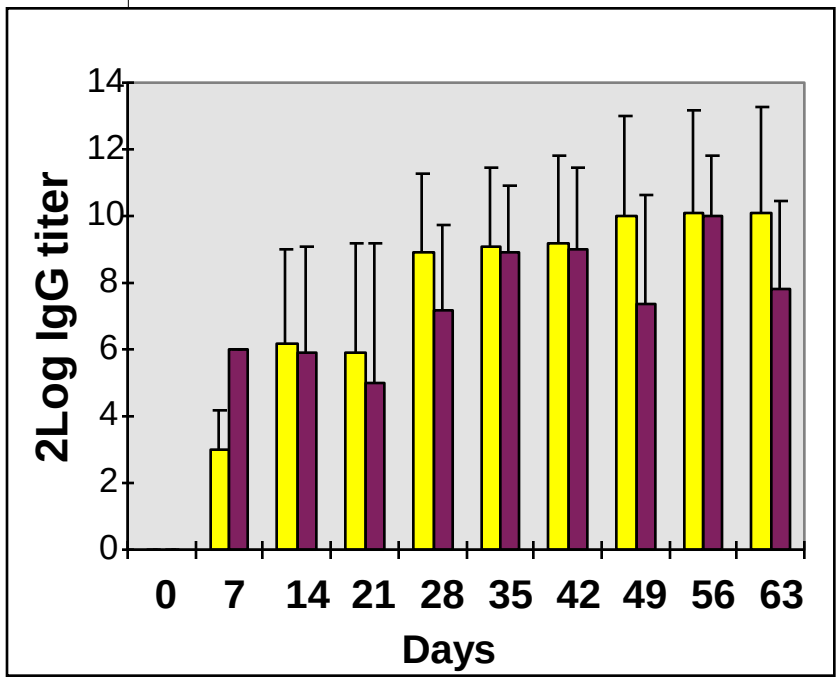
- Blood samples weekly
- IgG and IgE

protein by gavage

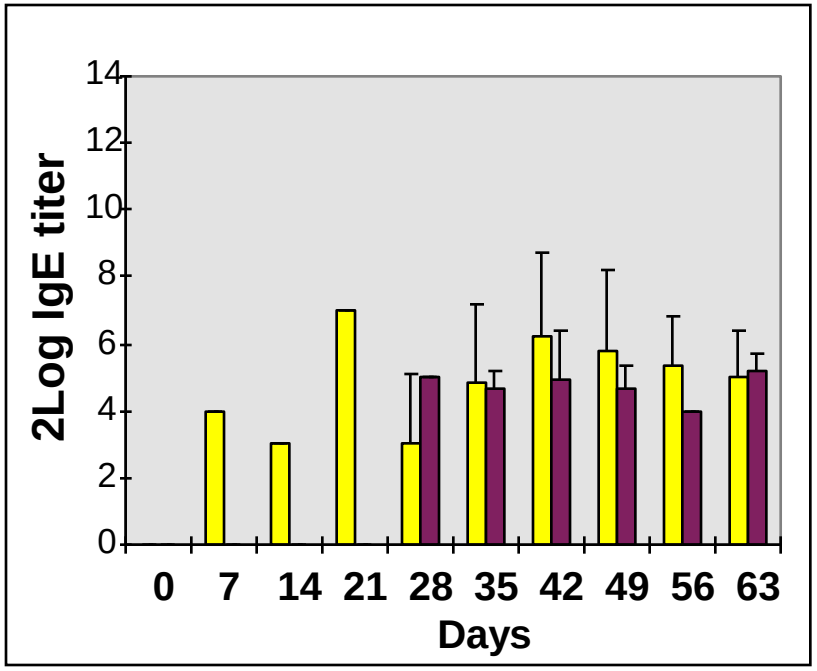
- Blood pressure
- Respiratory effects
- Gut permeability
- RMCPII
- T cell proliferation

Ovalbumin-specific IgG and IgE responses

Ovalbumin-specific IgG response

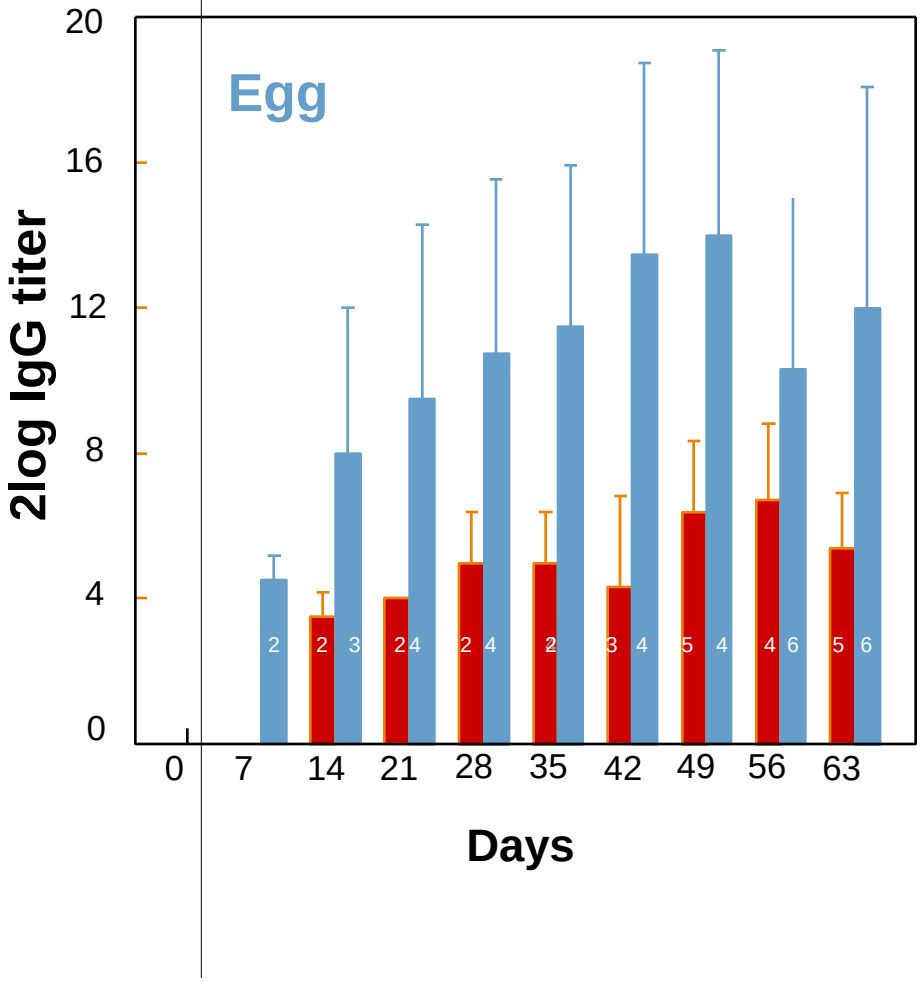


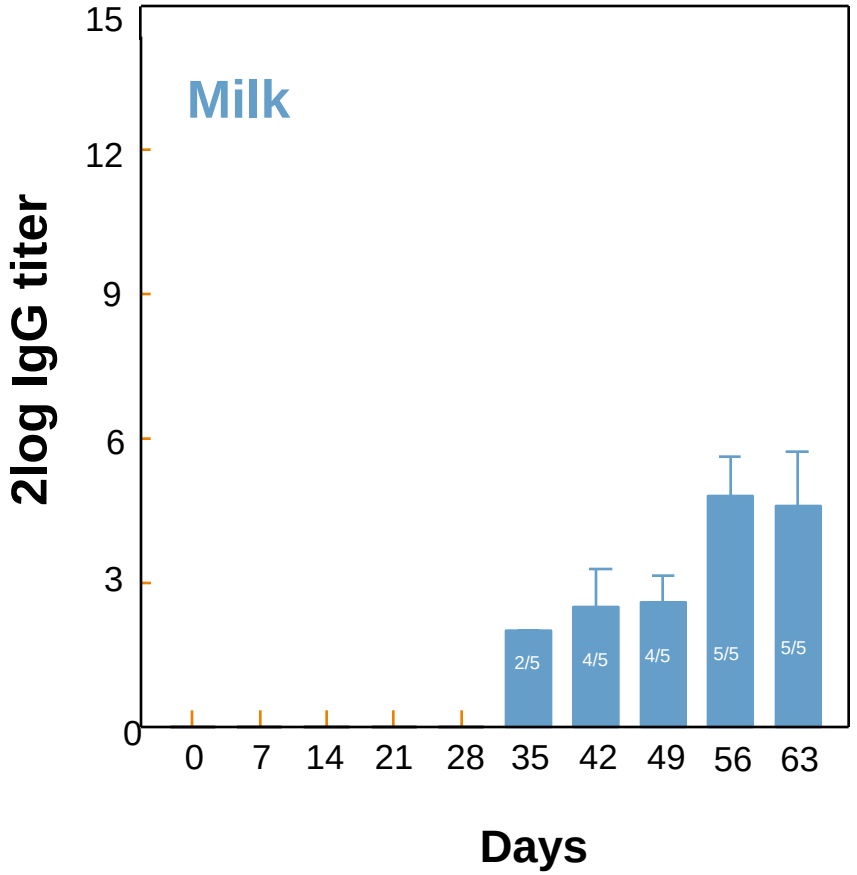
Ovalbumin-specific IgE response



Knippels et al, Allergy, 55, 251-258

Hen's egg white and cow's milk-specific IgG responses





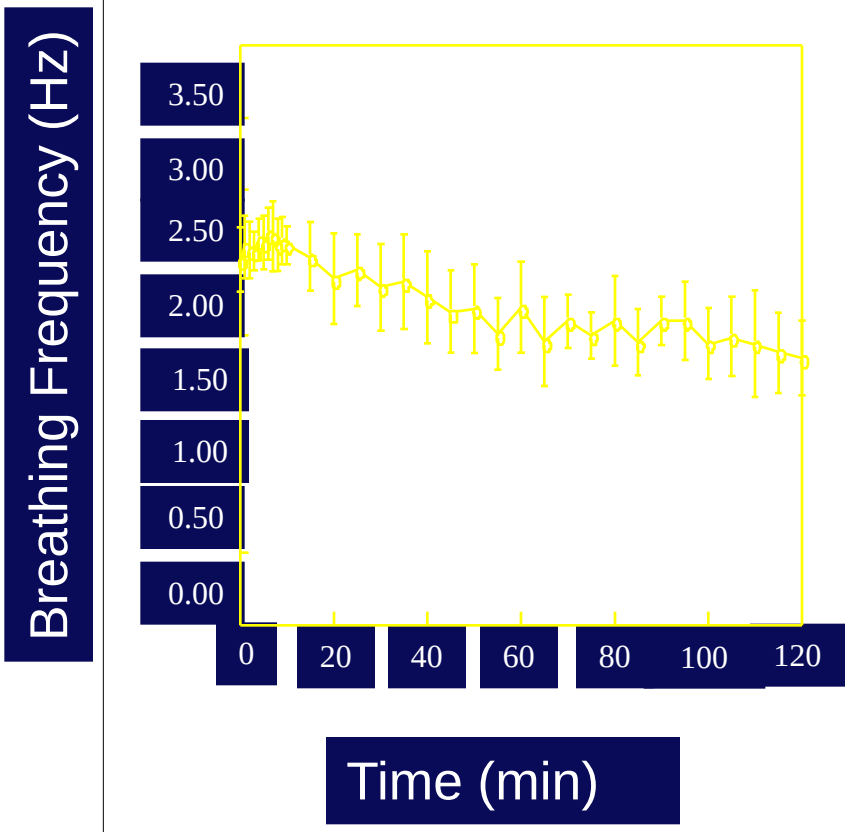
Knippels et al, Allergy, 55, 251-258

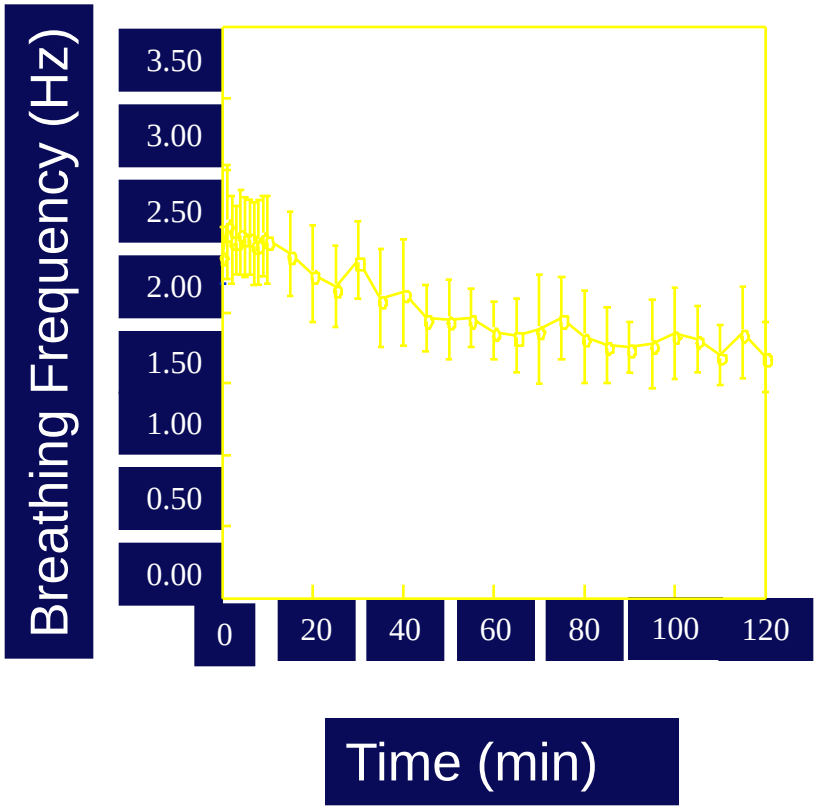
Immune-mediated clinical effects
(OVA sensitized and challenged)

- **Blood pressure** — **- decreased 10-20% of the sensitized animals**
- **Respiratory effects** — **- decreased 10-20% of the sensitized animals**
- **Gut permeability** — **- increased uptake bystander protein**
- **RMCP-II** — **- increased release in serum**
- **T cell proliferation** — **- increased**

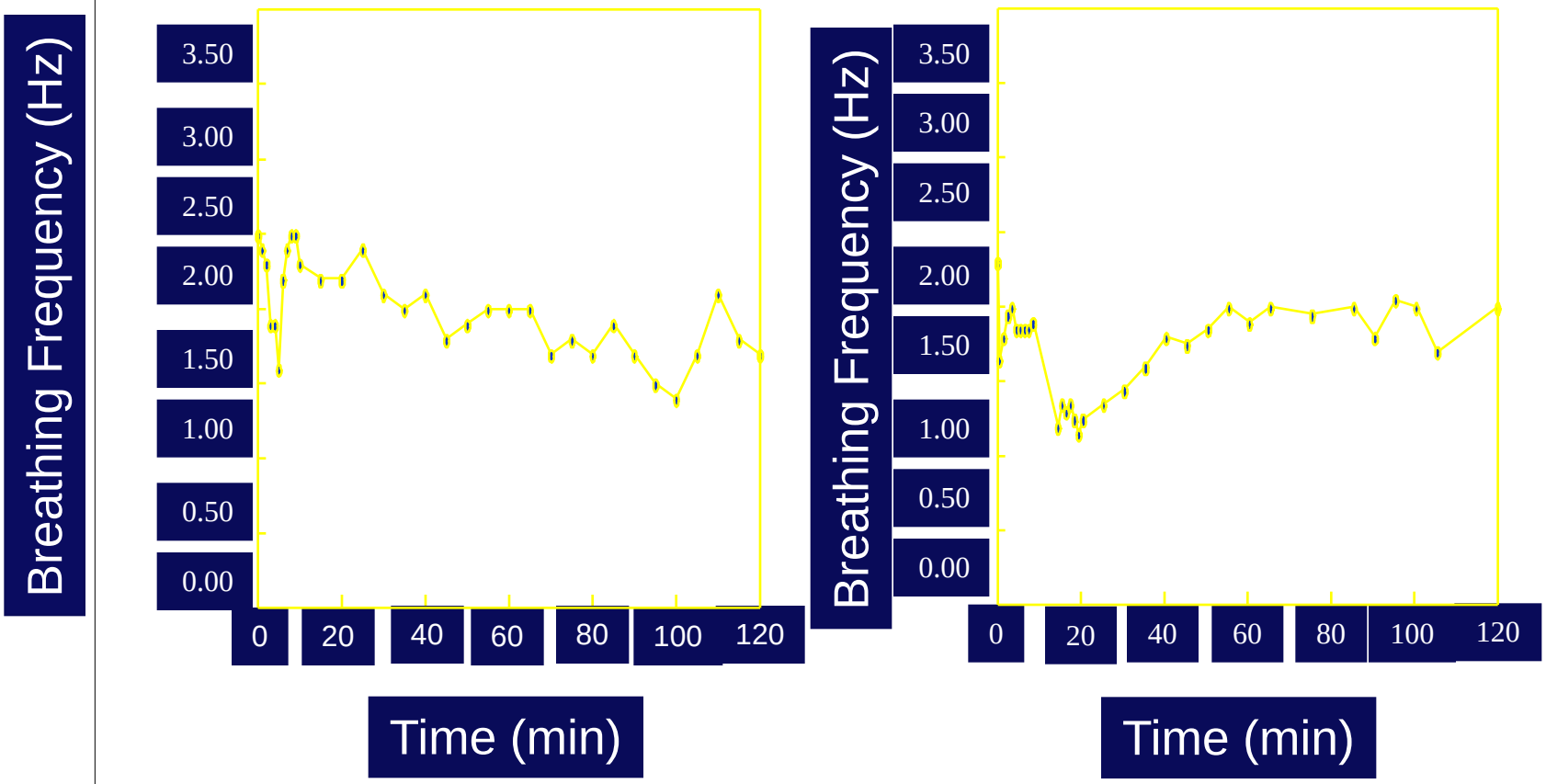
Knippels et al, Toxicol Appl Pharmacol, 1999, 156, 161-169

Airway responsiveness control animals





Airway responsiveness sensitized animals



β-lactoglobulin levels in sera from control and test animals

Time (hr)	0	0.5	1	2	3	5	8
ip sens.	<LOD	1.7-4.6*	0.9-2.4*	<LOD-1.6	<LOD	<LOD	<LOD
oral sens	<LOD	0.05-0.13*	0.07-0.09	<LOD-0.05	<LOD	<LOD	<LOD
control	<LOD	<LOD-0.02	<LOD	<LOD	<LOD	<LOD	<LOD

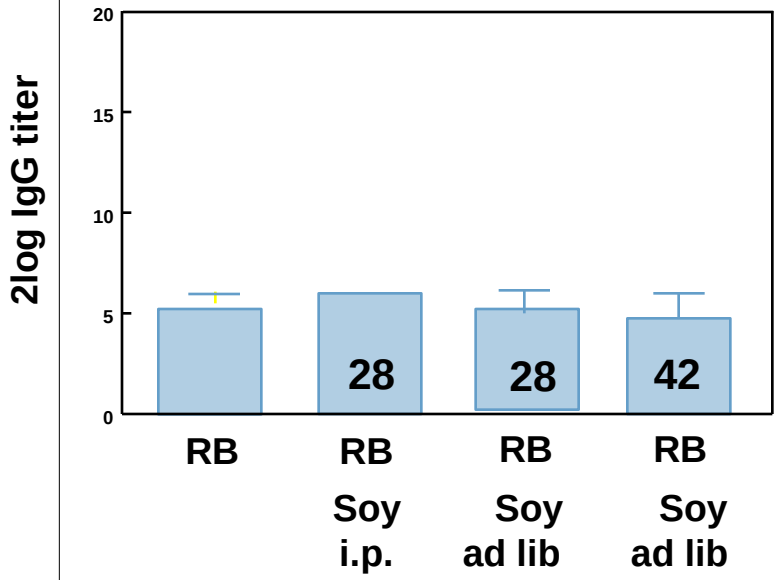
LOD: Limit of detection (0.01 µg/ml)

Knippels et al, Toxicol Appl Pharmacol, 156, 161-169

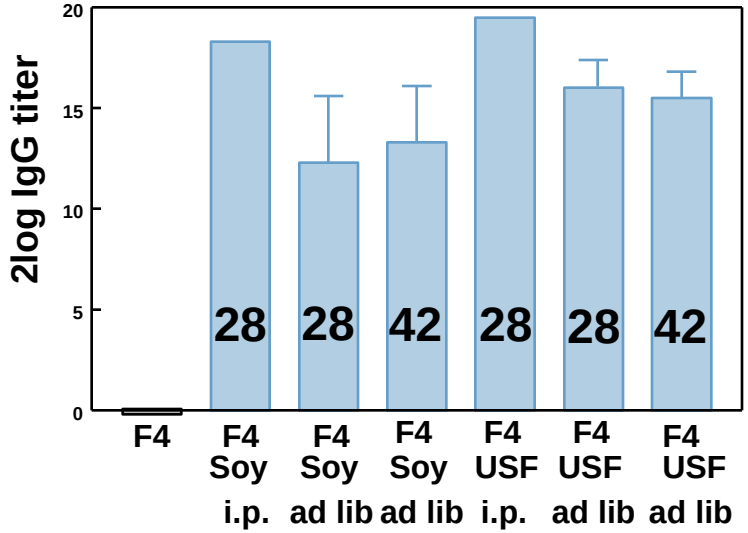
Unscheduled dietary pre-exposure!

Soy-protein specific IgG responses

rats bred on a soy-protein containing diet



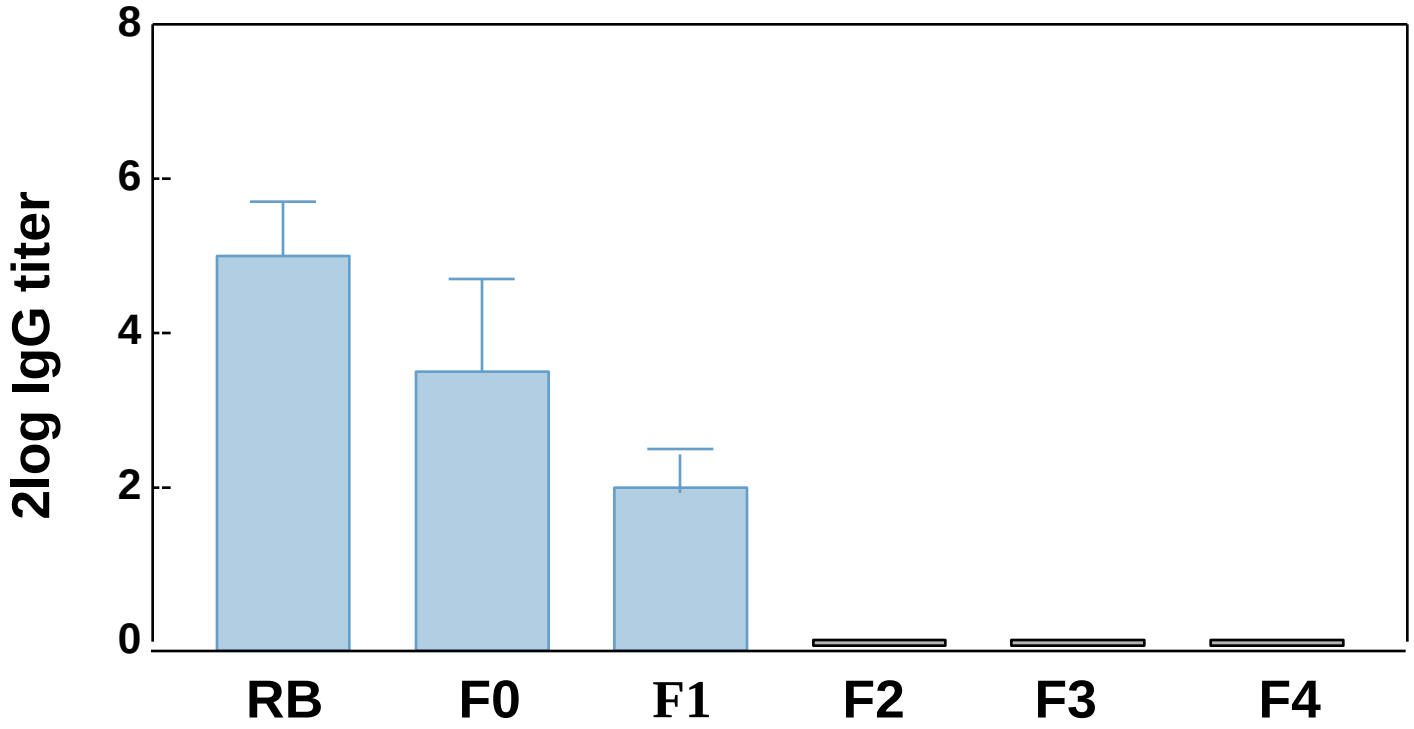
rats bred on a soy-protein free diet



i.p.

Knippels et al., JACI, 1998;101:815-20

Dietary Control
Soy-protein specific IgG titers



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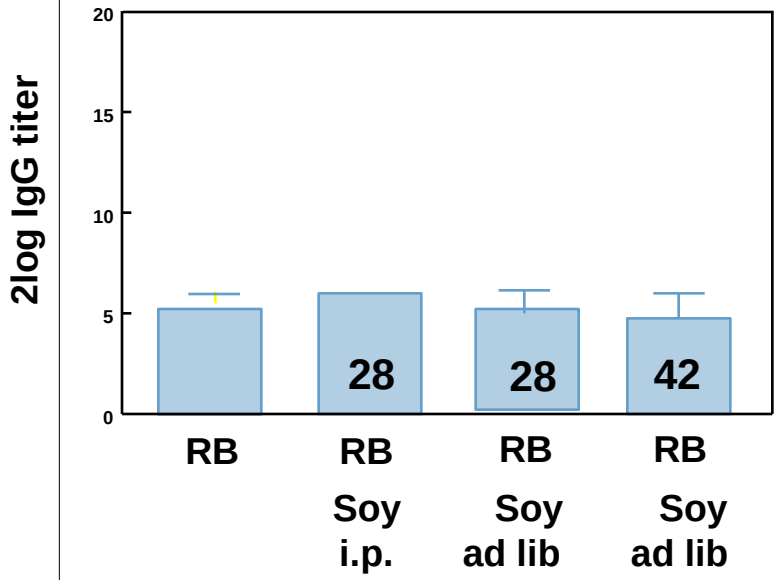
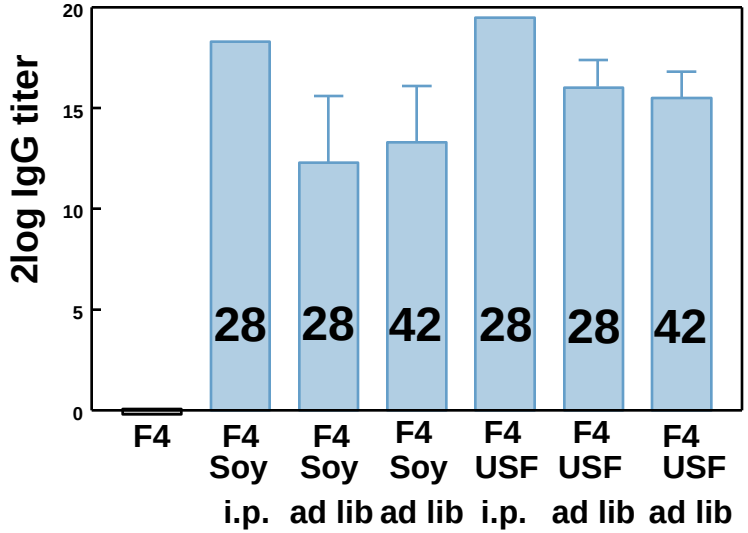
Soy-protein specific IgG responses

rats bred on a soy-protein containing diet

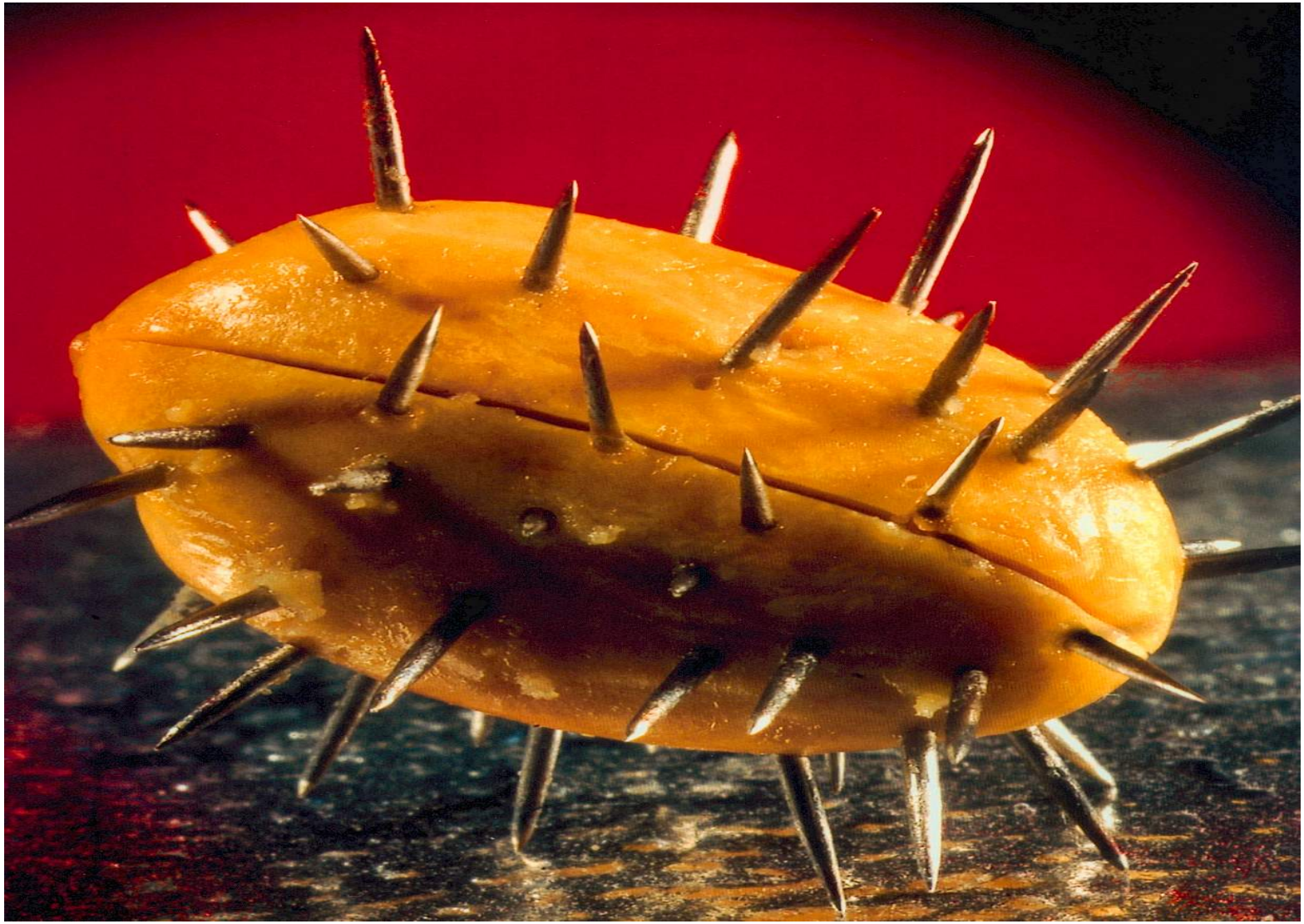


rats bred on a soy-protein free diet

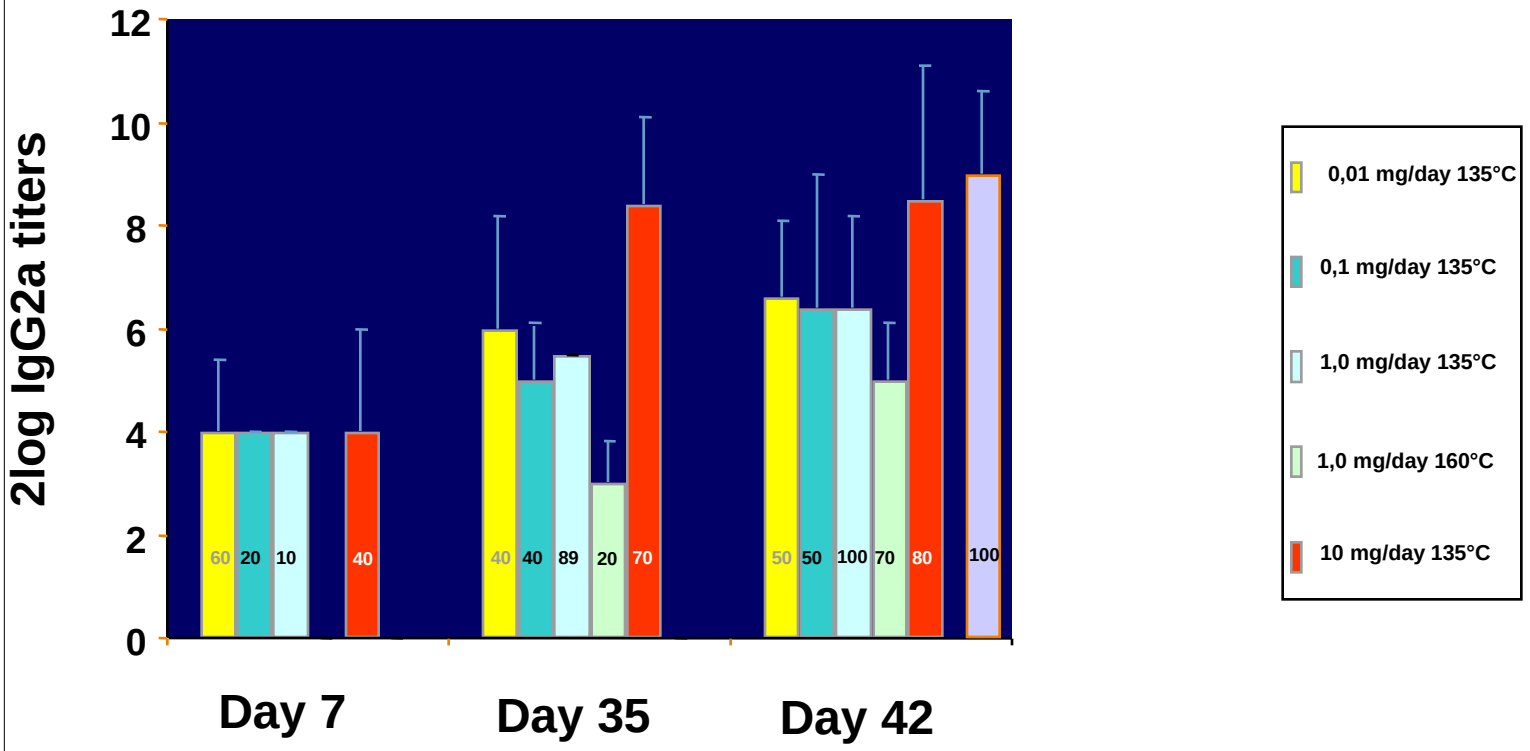


i.p.

Knippels et al., JACI, 1998;101:815-20

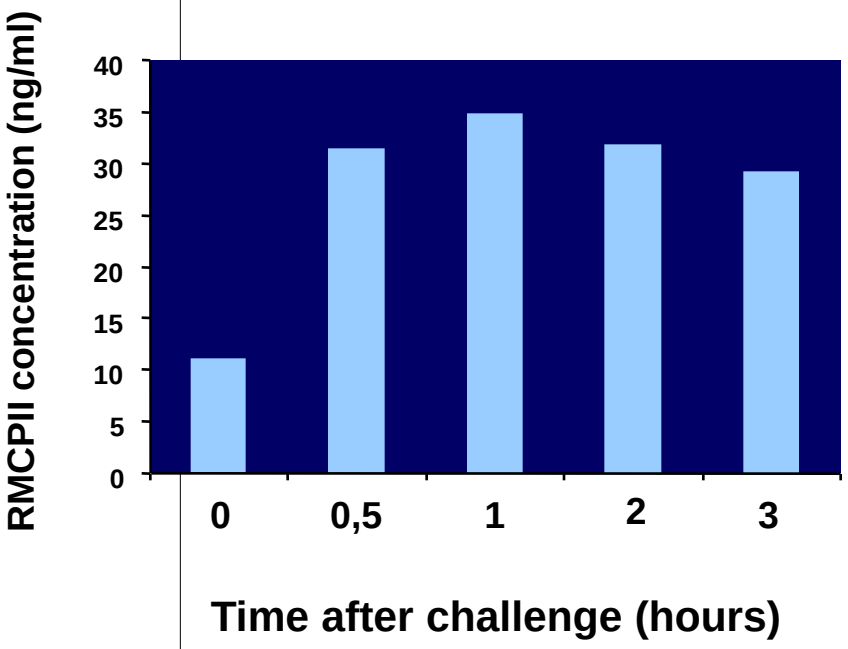


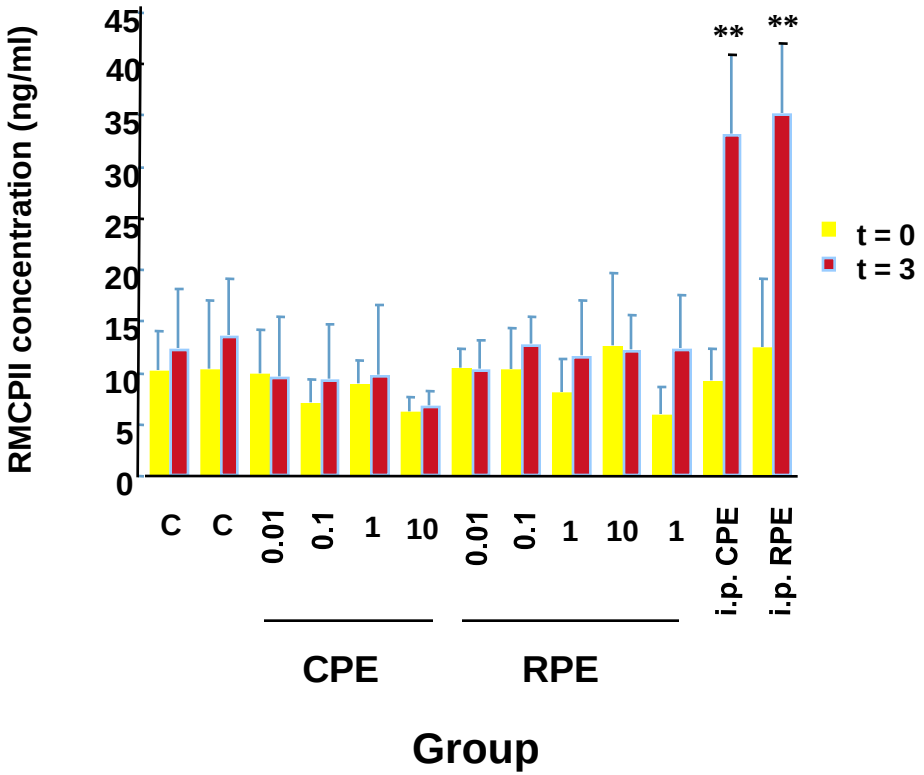
Peanut specific IgG2a responses



Ladics et al, Toxicol Sci, 2003; 73, 8-16
Kimber et al, Env Health Persp, 2003, 111, 8, 1125-1130

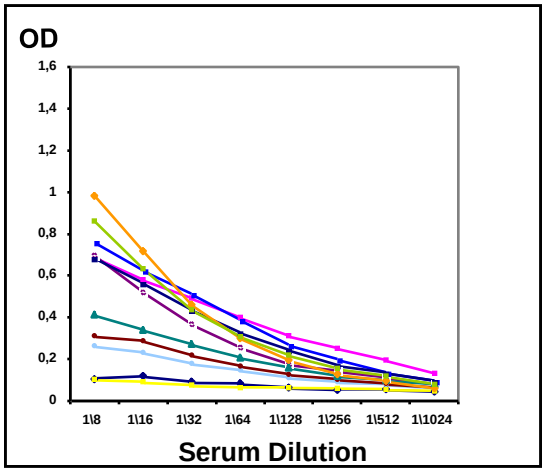
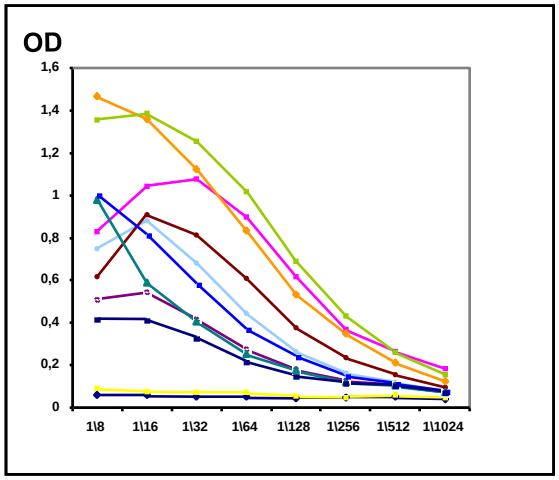
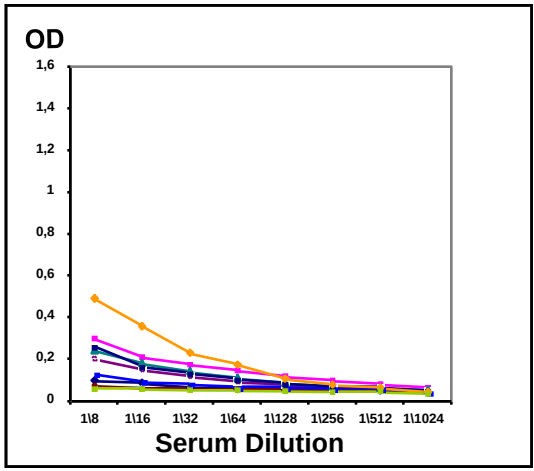
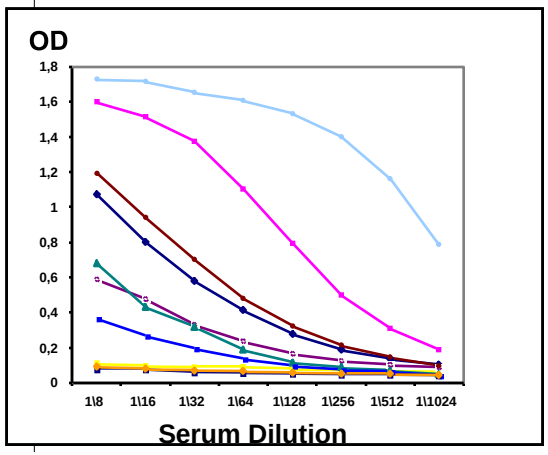
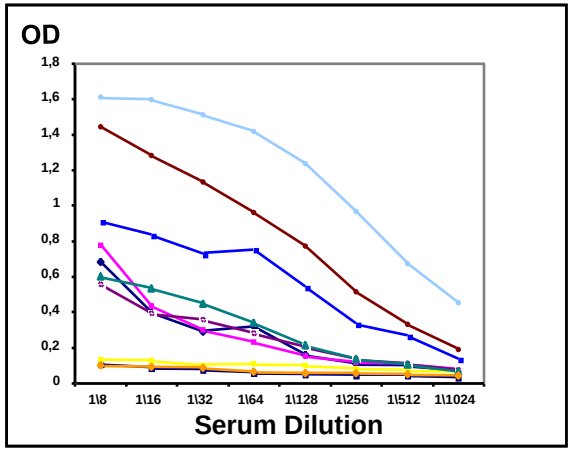
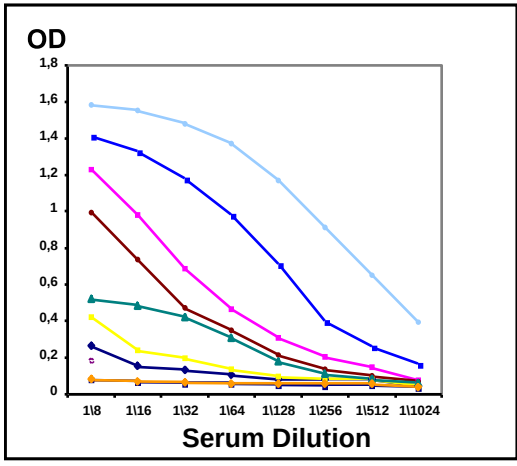
Rat mast cell protease II (RMCP-II) levels in serum after oral challenge with CPE or RPE





Specific IgG2a responses against Ara h1, Ara h2, and Ara h3

TNO triskelion bv

	Ara h1	Ara h2	Ara h3
I.P.			
Oral			

Ladics et al, Toxicol Sci, 2003: 73, 8-16; Ladics et al, Reg Tox Pharmacol, 2010, 56, 212-224

Dietary control in the development of a peanut allergy model in BN rats

- BN rats bred for 3 generations on a peanut- and soy- free diet (3G PE/soy-free) and rats raised on a commercially peanut-and whey-protein free diet (PE-W free) were daily i.g. dosed with 1 or 10 mg PE without adjuvant.
- In 3G PE/soy-free BN rats IgE titers > than those of the PE-W free rats
- In 3G PE/soy-free BN rats 100% IgE responders both to 1 and 10 PE.
- In PE-W free BN rats only 10% IgE responders (2/20) sensitized with 1 mg PE and no IgE responders when sensitized with 10 mg PE
- In 3G PE/soy-free BN rats 100% IgG1 and IgG2a responders and higher levels than in PE-W free BN rats
- In PE-W free BN rats 19/20 IgG1 and 17/20 IgG2a responders sensitized with 1 mg PE and 9/10 IgG1 and IgG2a responders sensitized with 10 mg PE

De Jonge et al, Methods 41,99-111, 2007

Purified Proteins

Protein	Source	Molecular weight (by gel-migration)	Purity
Sol t 1 (Patatin0 *Weak*	Potato tuber	40-43 kDa	92.6%
Ara h 1 *strong*	Peanut	60-67 kDa	88%
Shrimp tropomyosin *Intermediate*	Raw brown Shrimp	37-38 kDa	94.6%
Beef Tropomyosin *No allergen*	Raw beef	35-39 kDa	99.3%

Ladics et al, Reg Toxicol Pharmacol 2010, 56, 212-224

Brown Norway - IgE results – 2nd Exp.

Human allergen ranking

high

high

low-med.

non-

Day 42	IgE ELISA Titre(#rats)	Responders PCA # rats	IgG2a ELISA Titre	Responders ELISA # rats
Ara h1				
1 mg/ml	4.7 (3/6)	1/6	11.3	6/6
1 mg/ml + CT	4.3 (3/4)	0/4	4.5	2/4
I.p. + Alum	4.2 (6/6)	1/6	12.2	6/6
Pen a 1				
1 mg/ml	9.5 (2/6)	1/6	12.2	6/6
1 mg/ml + CT	0 (0/4)	0/4	10.5	4/4
I.p. + Alum	10.2 (6/6)	3/6	12.5	6/6
Sol t 1				
1 mg/ml	9 (1/6)	1/6	15	6/6
1 mg/ml + CT	8.5 (2/4)	0/4	14.5	4/4
I.p. + Alum	10.8 (6/6)	5/6	13.8	6/6
Beef				
1 mg/ml	0 (0/6)	0/6	3.75	4/6
1 mg/ml + CT	0 (0/4)	0/4	4	4/4
I.p. + Alum	0 (0/6)	0/6	3	3/6

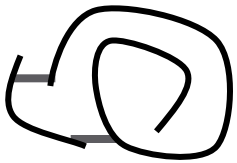
2S albumin form Brazil nut (Ber e1):

The role of -S-S- bridges

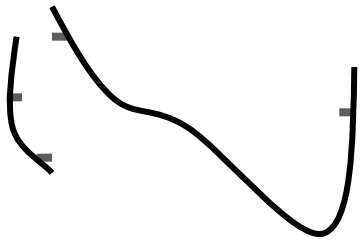
- **Hypothesis:**
  "Brazil nut 2S albumin is a potent allergen due to its stability towards digestion. This stability is supported by -S-S- bridges"

- **Testing the hypothesis:**
  prepare 2 forms of Brazil nut 2S albumin and test for: structure, stability, allergenicity

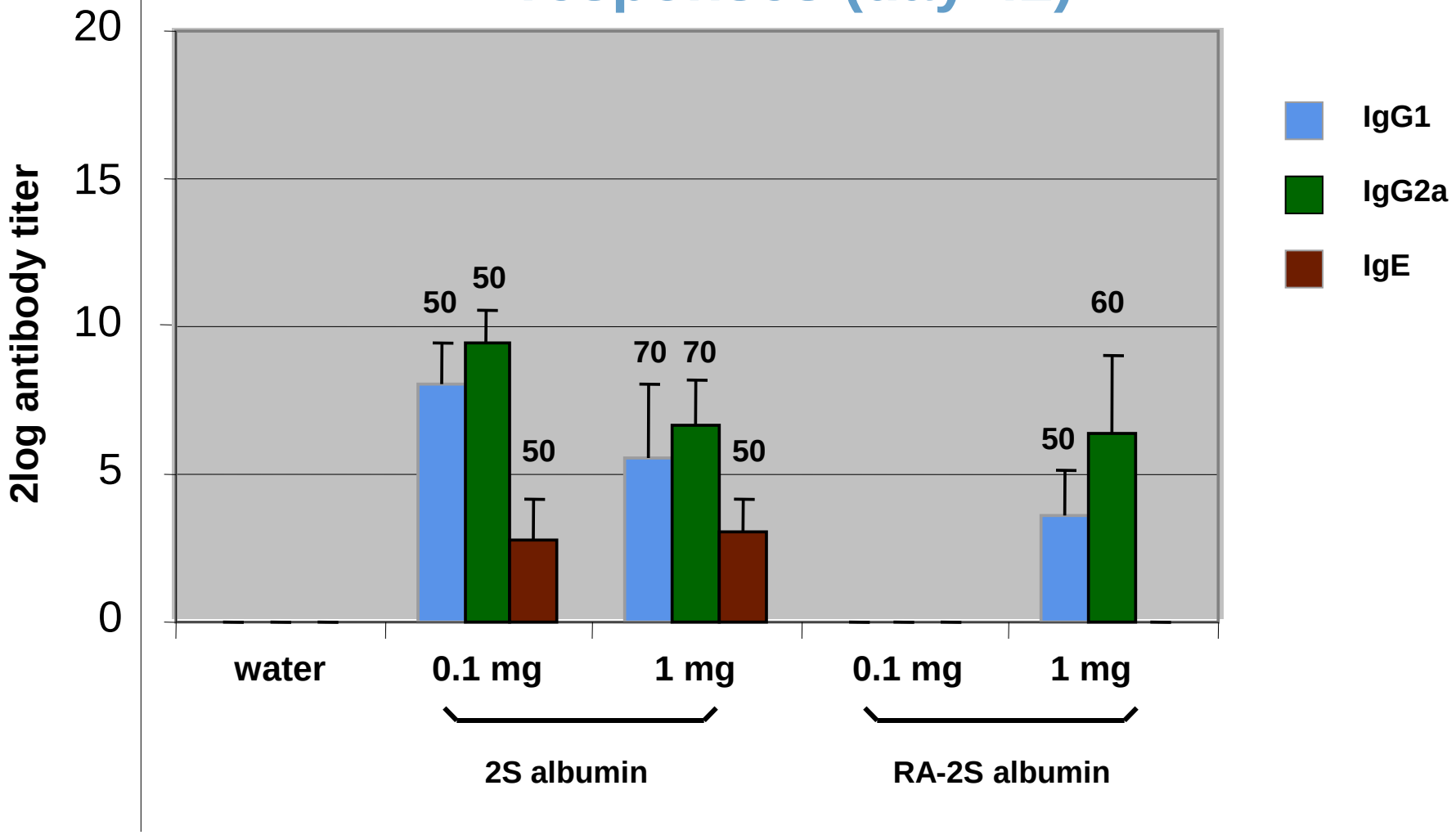
Native Brazil nut 2S albumin (N-2S)



Reduced-alkylated Brazil nut 2s albumin (RA-2S)



(RA)-2S albumin (Ber e1)-specific antibody responses (day 42)



IgG and IgE responses in BN rat model upon oral exposure to whole foods

Protein	^{2}log IgG	Number of responders	
		IgG	IgE
PE (1 mg)	10	10/10	4/10
HEW (2.5 mg)	7.2	5/6	1/6
HEW (10 mg)	14	4/6	2/6
CM (10 mg)	2.5	4/5	2/5

Knippels and Penninks, Toxicol Appl Pharmacol, 2005

IgG and IgE responses in BN rat model upon oral exposure to purified proteins

Protein	^{2}log IgG	Number of responders		
		IgG	IgE	i.p.
OVA	12.3	8/10	6/10	6/6
Ber e1	6.9	7/10	5/10	6/6
Ara h1	11.3	6/6	3/6	6/6
Pen a1	12.2	6/6	2/6	6/6
Sol t1	15	6/6	1/6	6/6
Beef tropo	3.8	4/6	0/6	0/6

Knippels and Penninks, Toxicol Appl Pharmacol, 2005

Results obtained with BN rats in other labs

- Atkinson et al, Toxicology 91, 281-288, 1994
- Atkinson et al, Fd Chem Toxicol 34, 27-34, 1996
- Miller et al, Clin Exp Allergy 29, 1696-1704, 1999

Diet, gavage and i.p. exposures in the presence of adjuvant

- Akiyama et al, Immunology Letters 78, 1-5, 2001

OVA-specific IgE upon gavage > drinking water (1 mg OVA/day)

- Jia et al, World J Gastroenterol 11, 5381-5384, 2005

IgE responders OVA (8/10) > BSA (2/10) > PAP (0/10)

- Madsen and Pilegaard, Int Arch Allergy Immunol 130, 66-72, 2003

No priming of the IR in newborn BN rats dosed with OVA in mouth

- Pilegaard and Madsen, Toxicology 196, 247-257, 2004

Female BN rats > IgE/IgG titers against OVA/egg white than males

- Bogh et al, Clin Exp Allergy 39, 1611-1621

Digested Ara h1 has sensitizing capacity in BN rats

Further Food Allergy Research (1)

- Currently at TNO no further development of the BN rat model for prediction of the potential allergenicity of new proteins.
- At TNO special attention will be given to the a slightly adapted protocol of the mice model of Li et al (1999, 2000) for assessment of potental allergenicity of new proteins.
- In collaboration with the Utrecht Centre of Food Allergy (UCFA) a new mice model is currently validated to test for hypo-allergenicity of partial and extensively hydrolysed infant formula (to replace the guinea pig ASA/PCA test).

Further Food Allergy Research (2)

- Mechanistic research in animal models for food allergy is further focussed on improvement of strategies for prevention (tolerance induction) and therapy.

- Special attention is given at TNO to the collection of physical and biological data of proteins (strong-, week-, and non allergenic proteins) for development of a toolbox to predict potential allergenicity of new proteins.

- Special attention is given at TNO to quantitative risk assessment in food allergy.

Dept Toxicology and Applied Risk Assessment
TNO Triskelion BV
Zeist

Thank you for your attention

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HQ in Zeist, The Netherlands

TNO Supervisory Board

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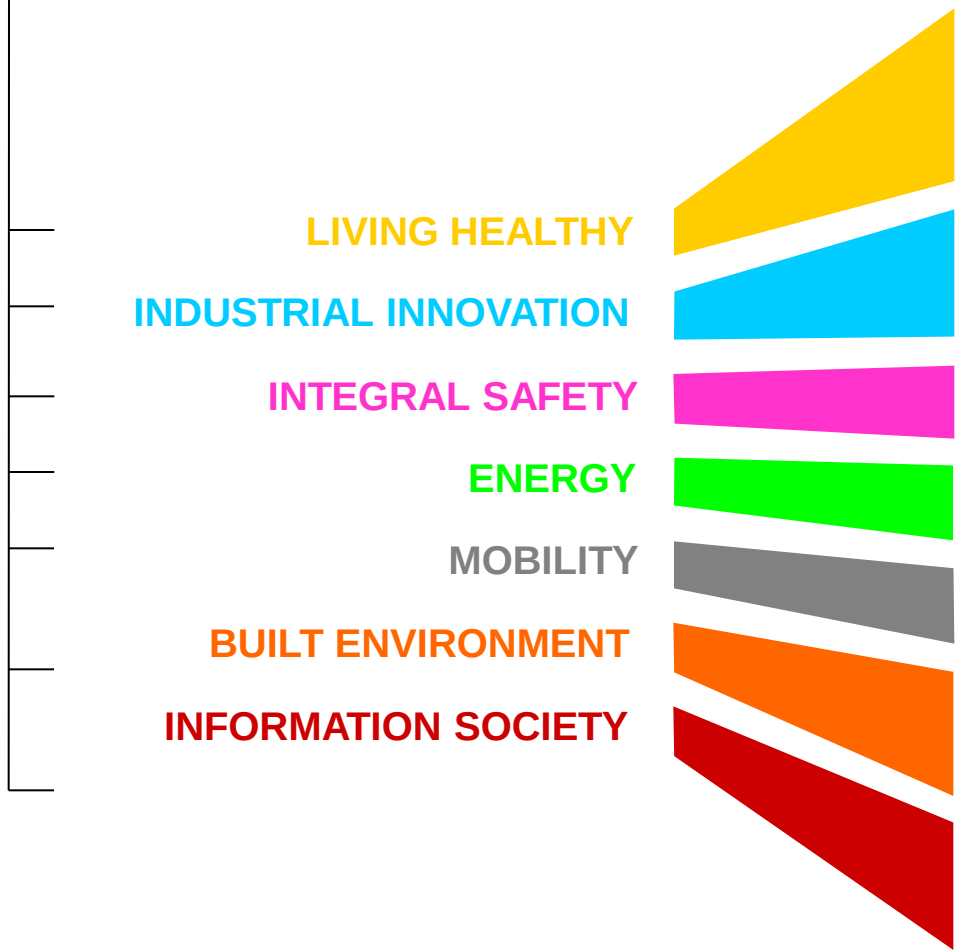
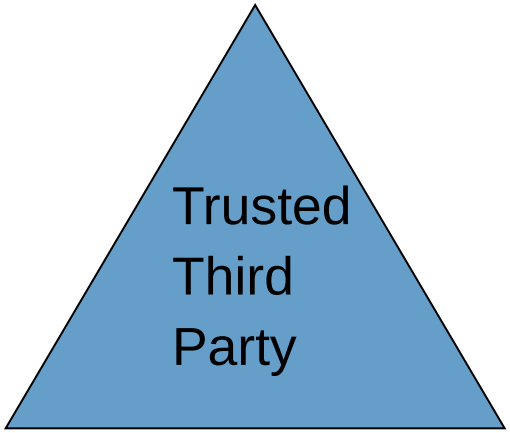


TNO Triskelion stands for:

Three markets
- Pharma
- Food and feed
- Chemistry



Three departments
- Analytical Research
- Toxicology and Applied Pharmacology
- Chemical Risk Assessment

Three components
- Quality
- Passion
- Operational excellence