MURINE MODELS FOR EVALUATING THE ALLERGENICITY OF GENETICALLY MODIFIED FOODS

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ILSI New Methods Workshop

23-25 October 2007

Nice – France.

Plan

- Critical parameters of animal models
- Mouse models
  - Literature review
  - ILSI ring trial (2005)
  - Syngenta-Bayer CS Collaborative work
  - A new promising mouse model
- Conclusion

Plan

- **Critical parameters of animal models**
- **Mouse models**
  - **Literature review**
  - **ILSI ring trial (2005)**
  - **Syngenta-Bayer CS Collaborative work**
  - **A new promising mouse model**
- **Conclusion**

Critical parameters for establishing a robust, cost effective and reproducible animal model

* Animals
  - Strain, age, sex, exposure to food, etc.
* Test material:
  - Allergens and non-allergens
  - Determination of matrix effect: pure protein *vs.* whole plant
  - Effects of food processing or digestion
  - Presence of adjuvants: modified immune responses
* Experimental design:
  - Route of administration (iv, ip, oral, td, etc.)
  - Frequency and dose of administration: graded allergenicity
* Biomarkers: IgE, IgG, cytokines, cell surface markers, etc.
* Reliability of the model: inter-laboratory comparisons
* Relevance to human data: (e.g. Active Systemic Anaphylaxis)

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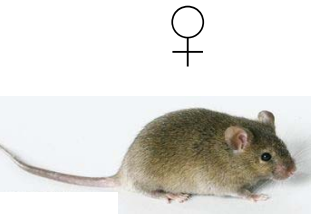
MOUSE MODELS OF FOOD ALLERGY

- X-M. Li et al. 2000 (Oral, Peanut)
- K. Adel-Patient et al. 2000 (I.P., Milk)
- R. Dearman et al. 2003 (I.P., OVA, BSA, PNA, STA, PAP)
- V. Morafo et al. 2003 (I.G., CM, PNA)
- C.J. Betts et al. 2004 (I.D., PNA & STA)
- F. vanWijk et al. 2004 (Oral, Peanut)
- S-Y. Lee et al. 2005 (I.G., Buckwheat)
- N. Birmingham et al. 2005 & 2007 (I.P. and T.D. , Hazelnut)
- K. Vaali et al. 2006 (T.D., OVA)
- Gizzarelli et al. 2006 (I.G., WT and GM-Soybean)
- etc…

Oral gavage sensitisation models



♀

4-8-w-old

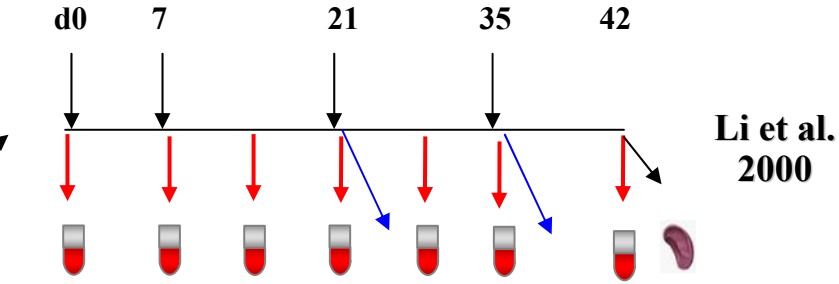
C3H/HeJ

Peanut Extract

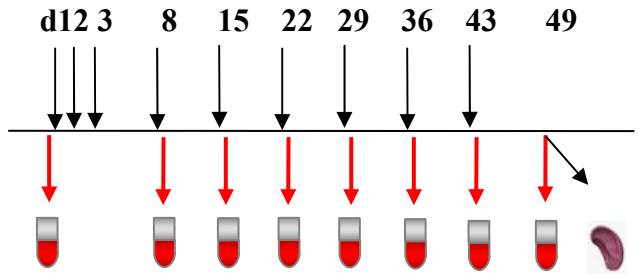
+ Adjuvant

Buckwheat Extract

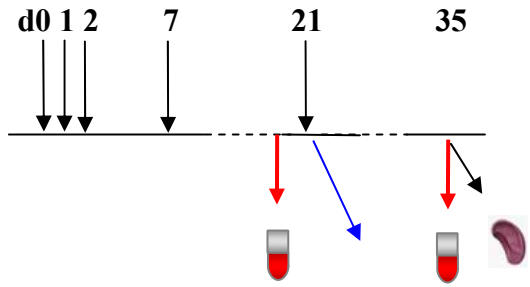
+ Adjuvant



Li et al. 2000



van Wijk et al. 2004



Lee et al. 2005

Oral gavage sensitisation models (continued)

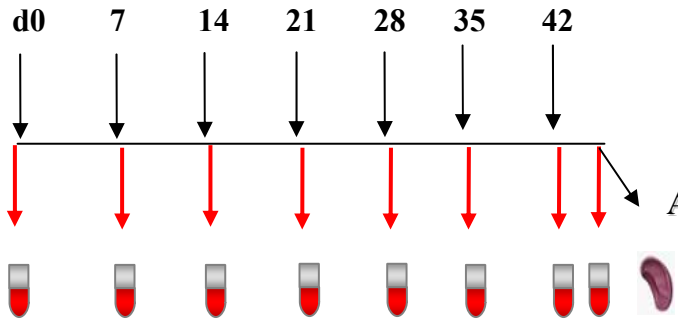
BALB/c

4-8-w-old

C3H/HeJ

Peanut or Milk

+ Adjuvant



Morafo et al. 2003

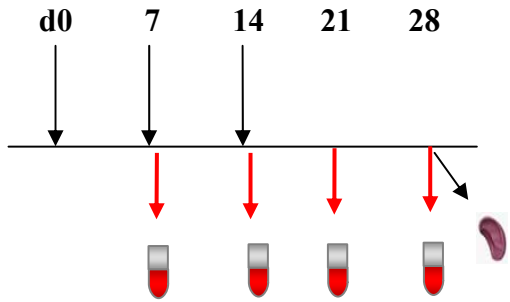
Adel-Patient et al. 2005

4-w-old

BALB/c

Wild type Soybean or GM-Soybean

+ Adjuvant



Gizzarelli et al. 2006

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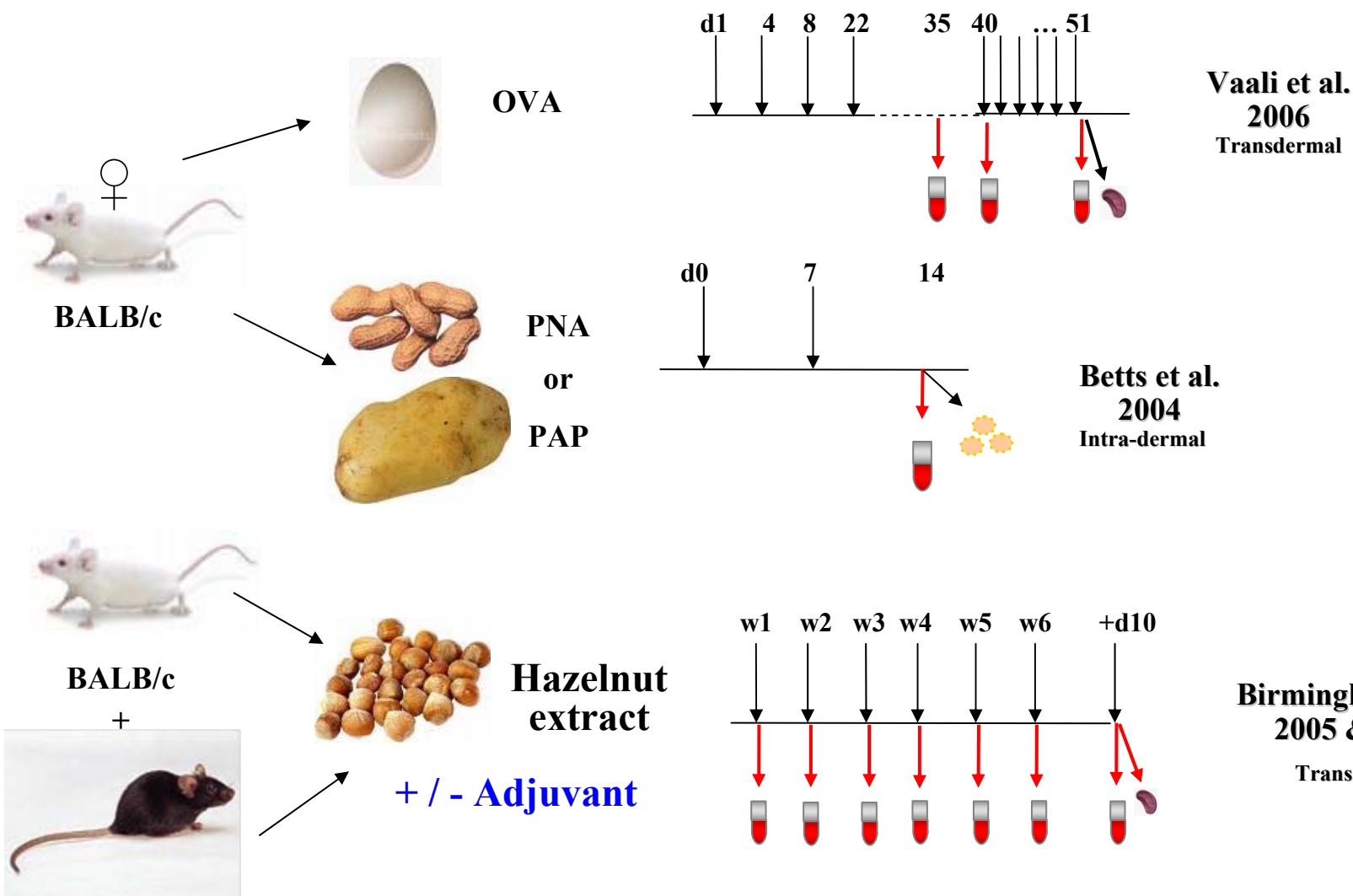
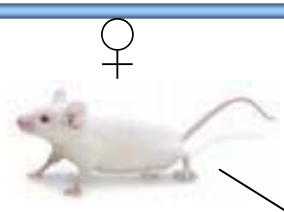
Dermal & Intra-dermal sensitisation models



I.P. sensitisation models

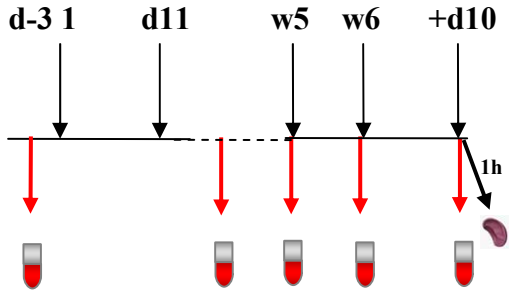


BALB/c

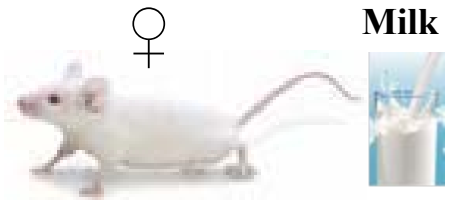
C57BL/6

Hazelnut extract

+ / - Adjuvant

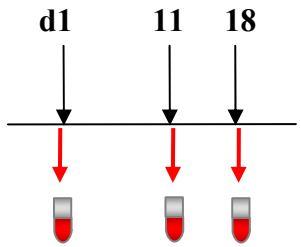


Birmingham et al. 2005



Milk

BALB/c

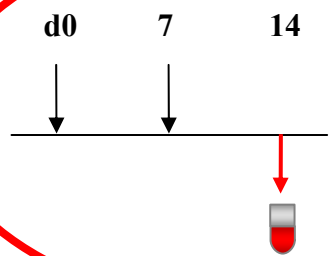


Adel-Patient et al. 2000

8-12-w-old

BALB/c

OVA, BSA, PNA, STA & PAP



Dearman et al. 2003

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Conclusion

- Many different models developed
- All based on IgE detection
- Adjuvant necessary for oral sensitisation
- Limited number of allergens and non-allergens tested

Plan

- Critical parameters of animal models
- Mouse models
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  - **ILSI ring trial (2005)**
  - Syngenta-Bayer CS Collaborative work
  - A new promising mouse model
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ILSI ring trial

Animal models

IP; no adjuvant; BALB/c
IP; no adjuvant; BALB/c, A/J, C3H
IP; no adjuvant; BALB/c
IP; +/- adjuvant; BALB/c, A/J, C3H, C57BL6
IP; +/- adjuvant; BALB/c

Laboratories

Bayer CropScience
Dupont
Syngenta
Monsanto
Dow

Test Proteins

Ara h 1 (peanut allergen)
Ara h 2 (peanut allergen)
B-lactoglobulin (cows' milk allergen)
Rubisco (non-allergen)
Soy lipoxygenase (non-allergen)

ILSI ring trial

Endpoints measured:

Clinical scoring => Active Systemic Anaphylaxis (ASA)

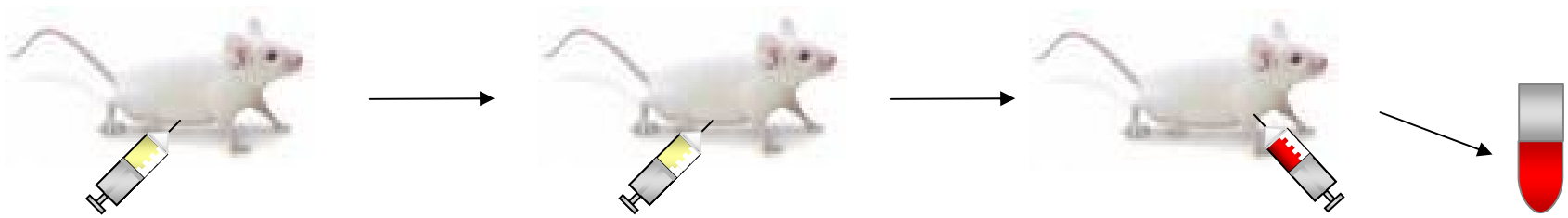
IgG levels => ELISA

IgE levels => Passive Cutaneous Anaphylaxis (PCA)

Passive Cutaneous Anaphylaxis (PCA)
Phase I: Sensitisation

Protein i.p. injections **Blood withdrawal**

D0 **D7** **D14**



Naïve animal **Sensitised animals**

IgG

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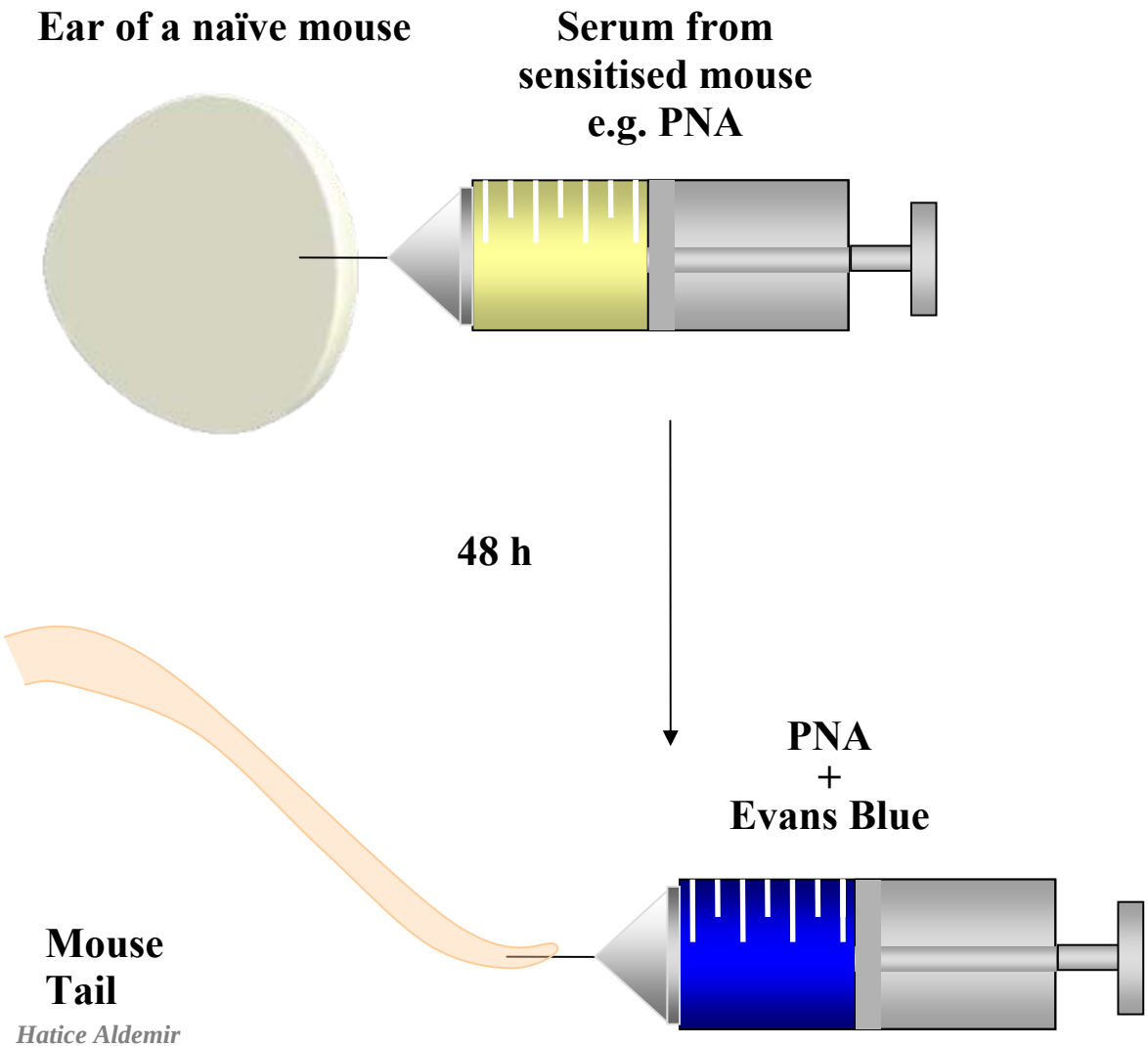
Passive Cutaneous Anaphylaxis (PCA) Phase II: Elicitation



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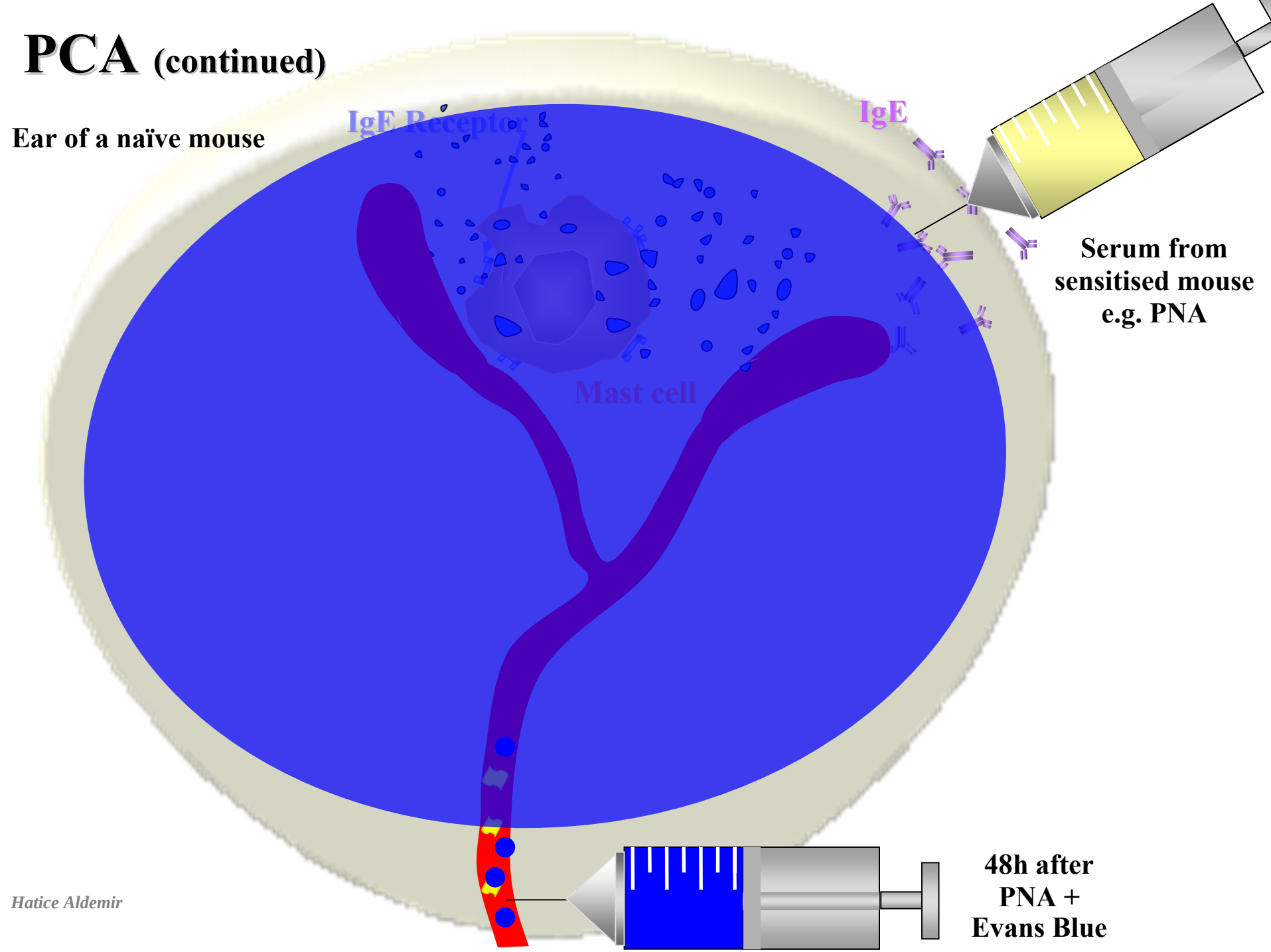
PCA (continued)



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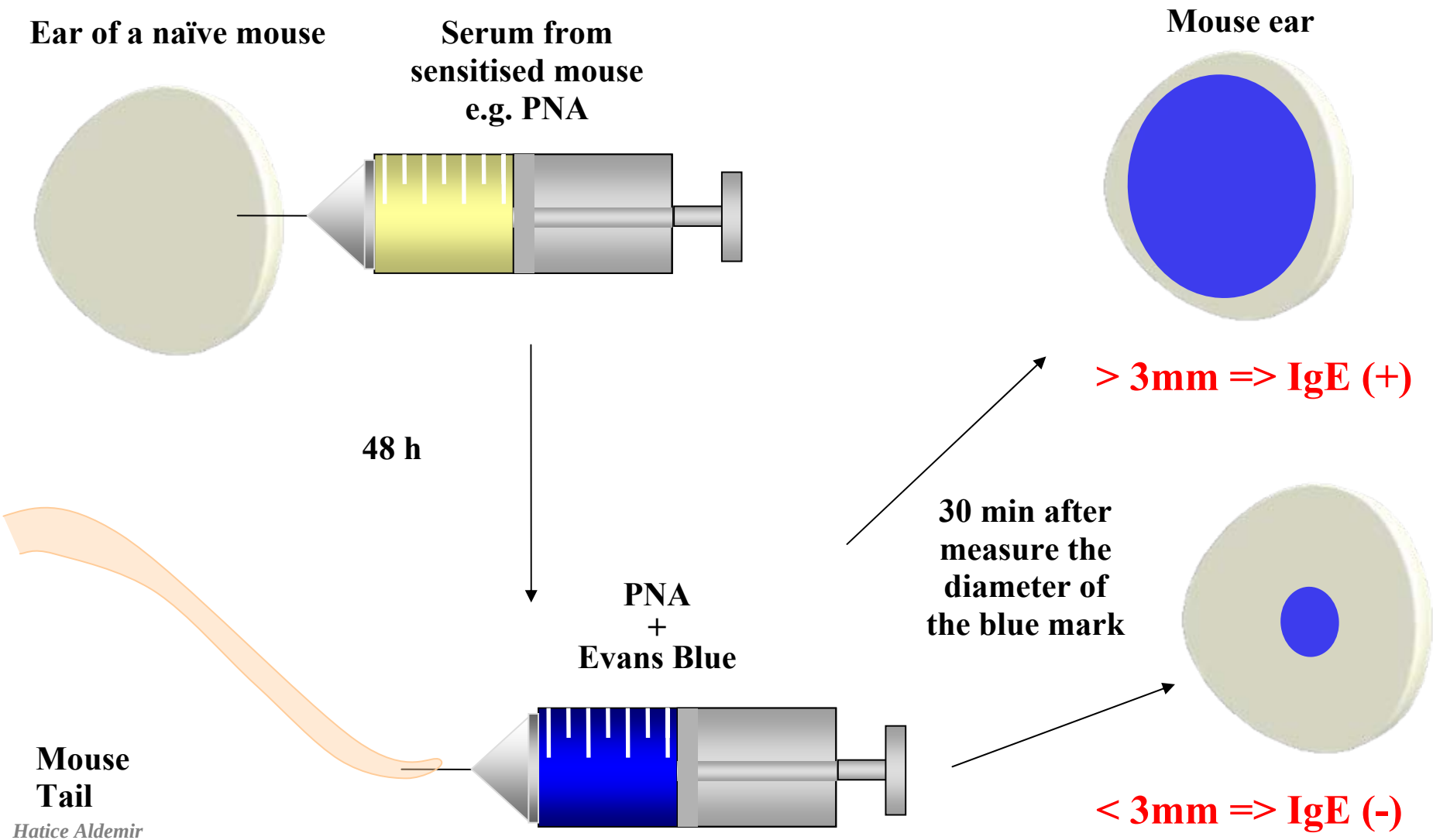
Passive Cutaneous Anaphylaxis (PCA)
Phase II: Elicitation



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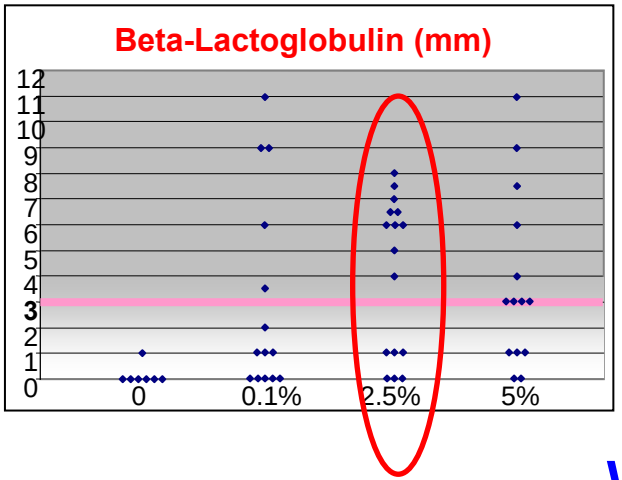
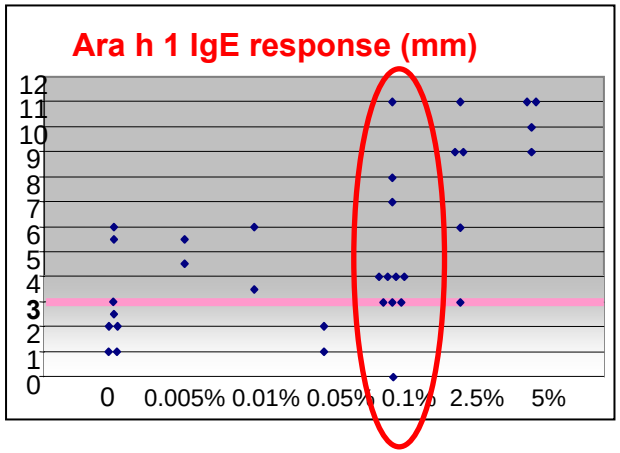
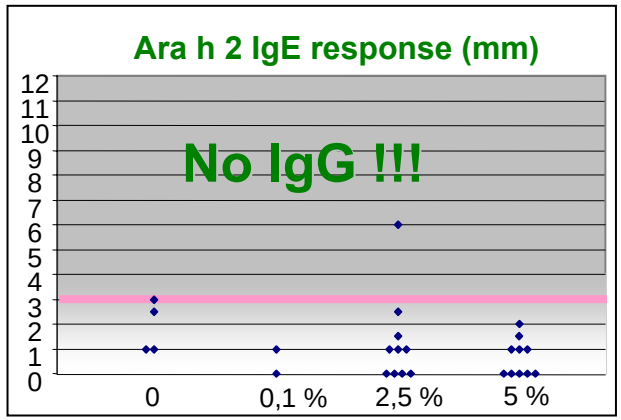
BCS Results

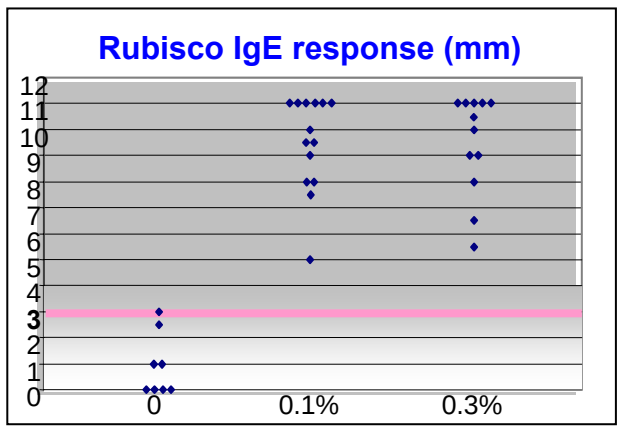
STRONG HUMAN ALLERGENS

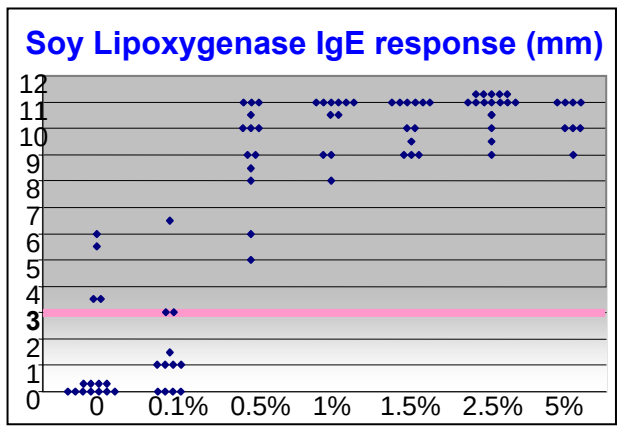






WEAK HUMAN ALLERGENS





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But high LPS contamination and potential cross-reactivity!

Combined Results

Protein	Lab A Sensitize days 1, 7, 14 IgG => ELISA IgE => PCA d21		Lab B Sensitize days 1, 7 IgG => ELISA IgE => PCA d14		Lab C Sensitize days 0, 7, 28 IgE => het. PCA day 28 Clinical Symptoms d45		Lab D Sensitize days 1, 7 IgG =>ELISA IgE => PCA d14
	BALB/c	BDF1	C3H/HeJ	BDF1	A/J	C3H/HeJ	BALB/c
Ara h1	(+) specific IgE	(+) specific IgE	Not examined.	Not examined.	Positive Clinical Score	Positive Clinical Score	(+) specific IgG and IgE
Ara h2	(-) specific IgG and IgE	(-) specific IgG and IgE	Not examined.	Not examined.	Positive Clinical Score	Positive Clinical Score	(-) specific IgG
β-lacto-globulin	(-) specific IgE	(-) specific IgE	Not examined.	(+) specific IgG and IgE	Negative Clinical Score	Negative Clinical Score	(+) specific IgG and IgE
Rubisco	(+) specific IgE	(+) specific IgE	(+) specific IgG and IgE	Not examined.	Negative Clinical Score	Negative Clinical Score	(+) specific IgG and IgE
Soybean Lipo-oxygenase	(+) specific IgE	(+) specific IgE	(+) specific IgG and IgE	Not examined.	Positive Clinical Score	Positive Clinical Score	(+) specific IgG and IgE

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EVALUATION OF IP MOUSE MODELS FOR ASSESSING THE ALLERGENIC POTENTIAL OF PROTEINS. Thomas, K., Hérouet, C., Bannon, G., Ladics, G., MacIntosh, S., Privalle, L., Woolhiser, M., Hefle, S. **ILSI, Bayer CropScience, Monsanto, Dupont Co., Bayer Bioscience, BASF, The Dow Chemical Company, University of Nebraska. AAAAI Congress, San Antonio, 2005.**

ILSI ring trial: Conclusion

- **Difficult to differentiate between allergens and non-allergens consistently with various models.**
- **Adequate purification (e.g. LPS) and good characterization of proteins are important.**
- **The mechanisms of action for each protein (allergy *vs.* tolerance) need to be explored.**

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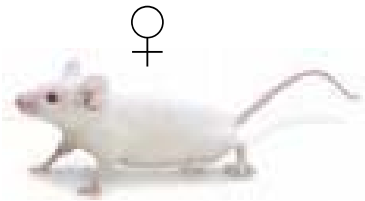
R.J. Dearman et al. => Intraperitoneal sensitisation model 2003



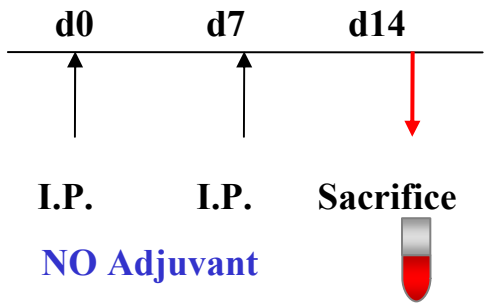
8-12-w-old

BALB/c

Ovalbumin

Peanut agglutinin

Potato agglutinin

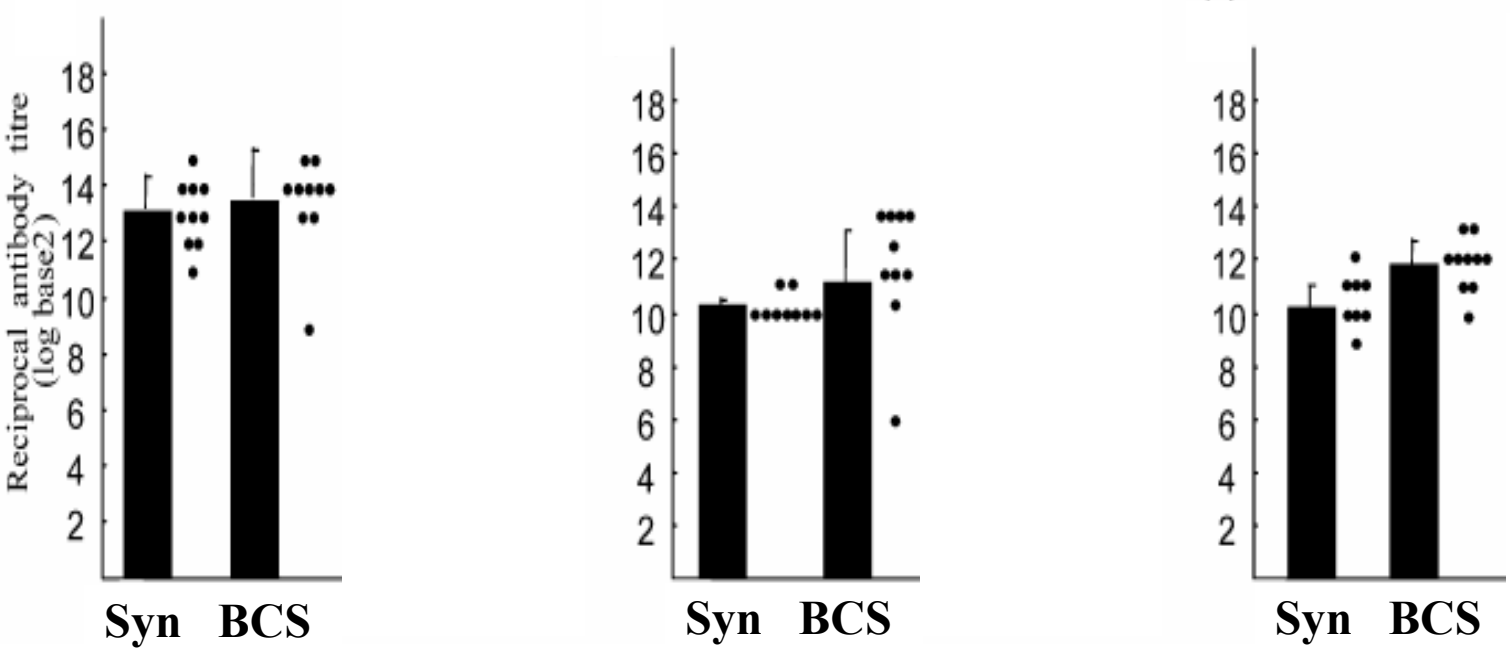


Dearman RJ, Stone S, Caddick HT, Basketter DA, Kimber I. Evaluation of protein allergenic potential in mice: dose-response analyses. Clin Exp Allergy. 2003 Nov;33(11):1586-94.Clinical and Experimental Allergy 33: 1586, 2003.

Results: Phase I

2003

Serum levels of specific-IgG ■ and IgG1 ●



PNA 0.1%
Peanut

2% OVA
Egg

5% STA
Potato

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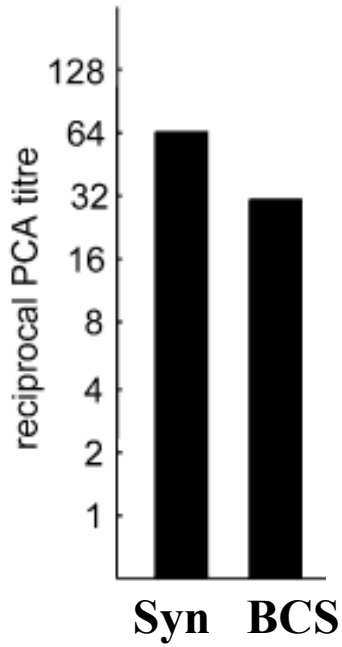
Dearman RJ, Skinner RA, Herouet C, Labay K, Debruyne E, Kimber I. Induction of IgE antibody responses by protein allergens: inter-laboratory comparisons. Food Chem Toxicol. 41(11): 1509-1516, 2003.

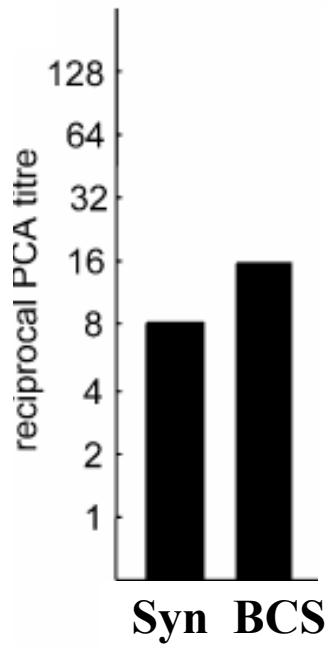
Results: Phase I

2003

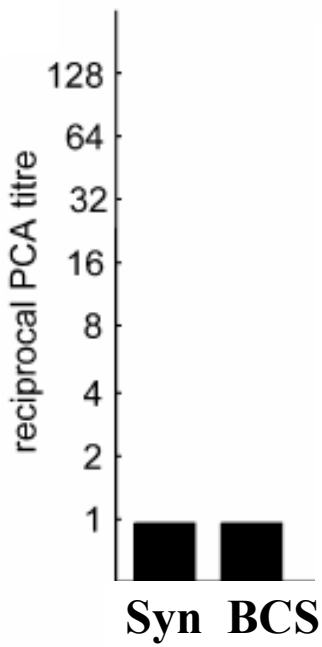
Serum levels of specific IgE



PNA 0.1%
Peanut



2% OVA
Egg



5% STA
Potato

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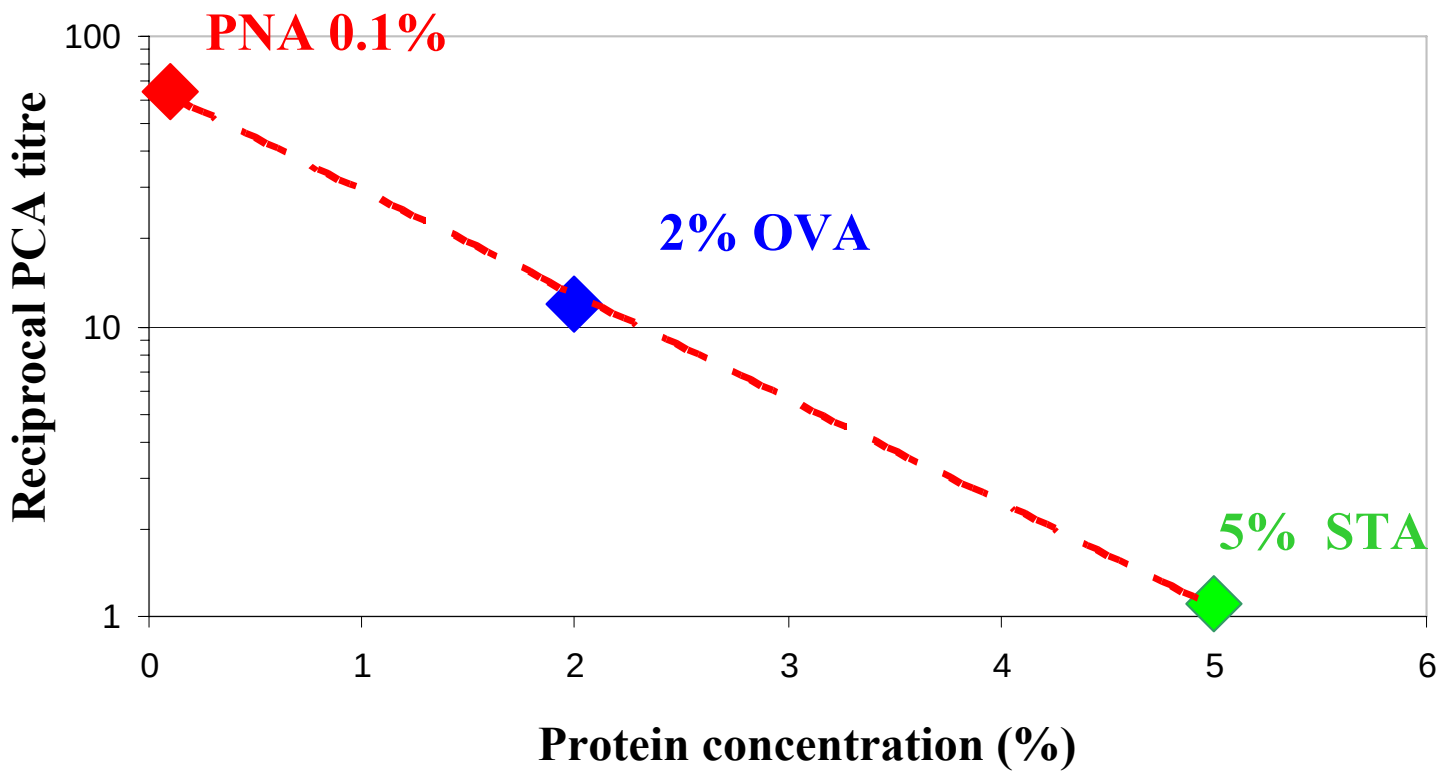
Dearman RJ, Skinner RA, Herouet C, Labay K, Debruyne E, Kimber I. Induction of IgE antibody responses by protein allergens: inter-laboratory comparisons. Food Chem Toxicol. 41(11): 1509-1516, 2003.

Apparent spectrum based on IgE levels



Conclusions: Phase I

2003

=> Measurement of IgE responses by PCA in BALB/c mice appears to accurately identify allergens.

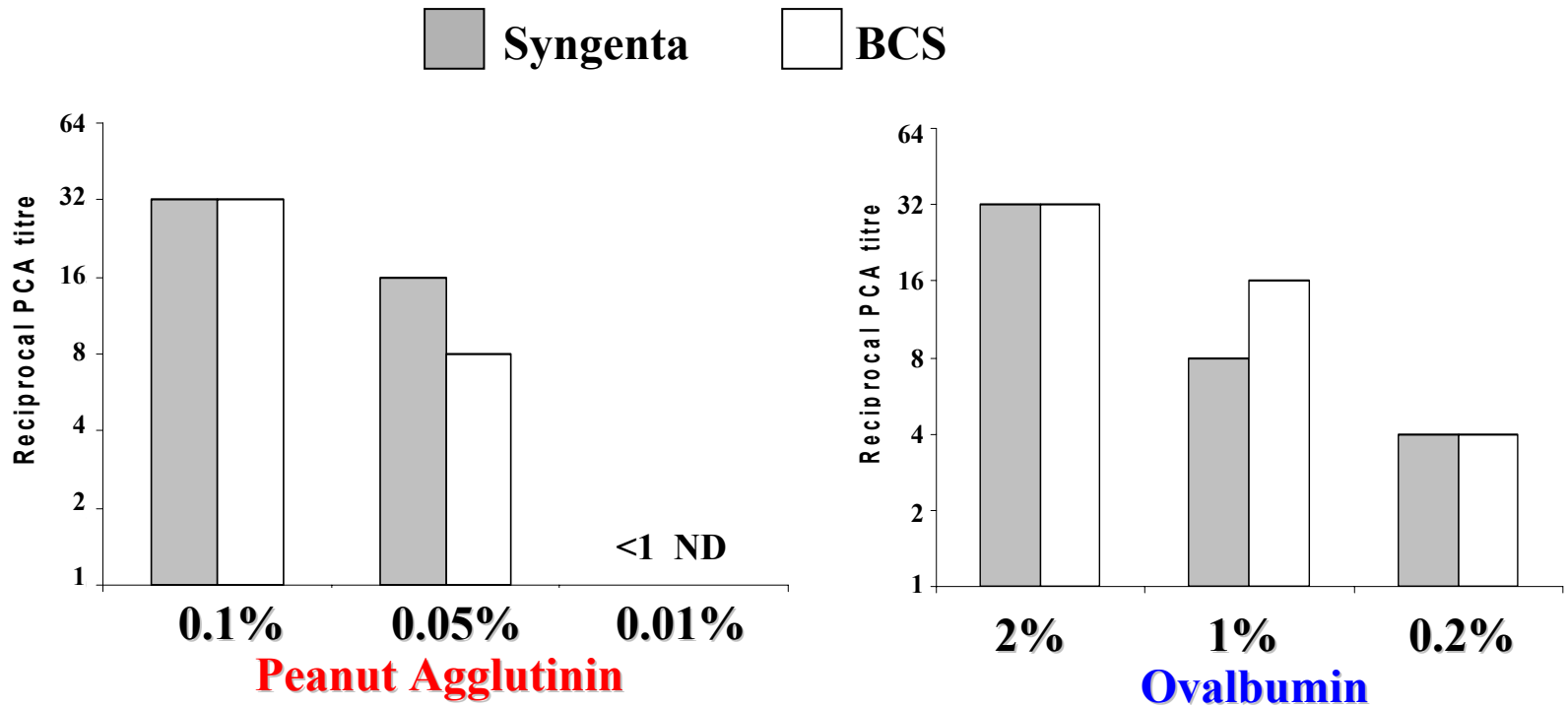
=> This model is relatively robust and can be transferred successfully between laboratories.

Dearman RJ, Skinner RA, Herouet C, Labay K, Debruyne E, Kimber I. Induction of IgE antibody responses by protein allergens: inter-laboratory comparisons. Food Chem Toxicol. 41(11): 1509-1516, 2003.

Results: Phase II

2007

IgE levels



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C. Herouet-Guicheney, H. Aldemir, R. Bars, D. de Barbeyrac, D. Rouquié, P. Kennel, B. Stahl, R. Dearman, I. Kimber.
Manuscript ready for submission.

Conclusions: Phase II

2007

=> Confirmation of the strength and repeatability of the model.

=> The model needs to be standardized by testing more proteins.

C. Herouet-Guicheney, H. Aldemir, R. Bars, D. de Barbeyrac, D. Rouquié, P. Kennel, B. Stahl, R. Dearman, I. Kimber.
Manuscript ready for submission.

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Aim

A new model of food allergy

Strongly allergenic

Weakly allergenic

Peanut

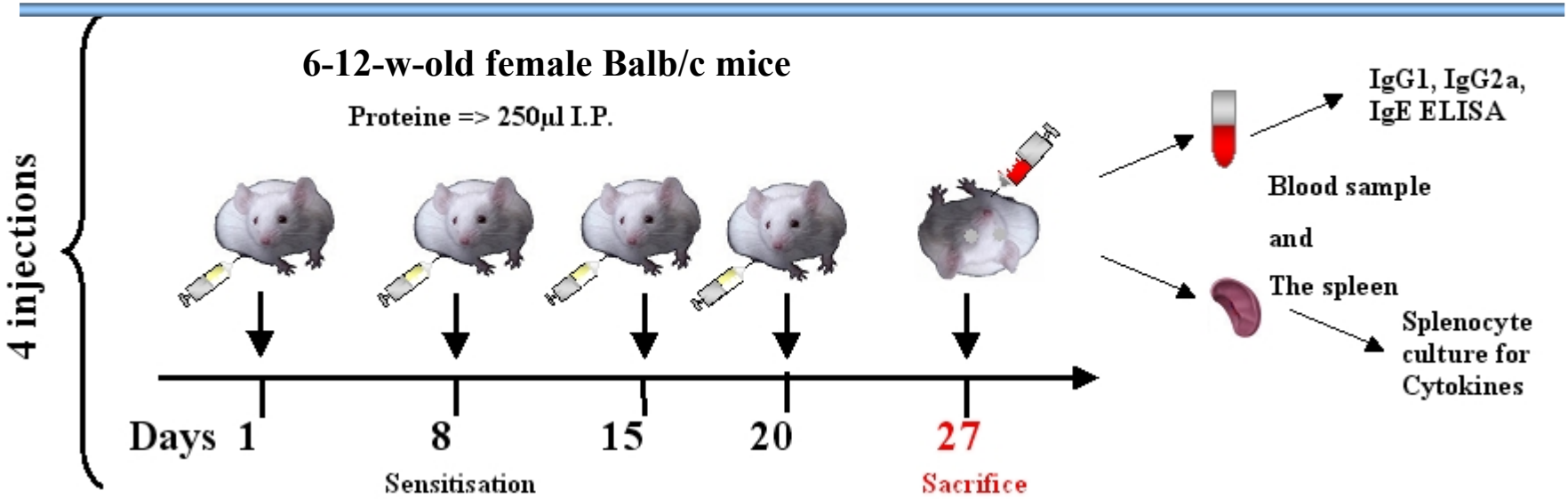
Ara h 1

Peanut Agglutinin

Potato

PAP

Sensitisation protocol

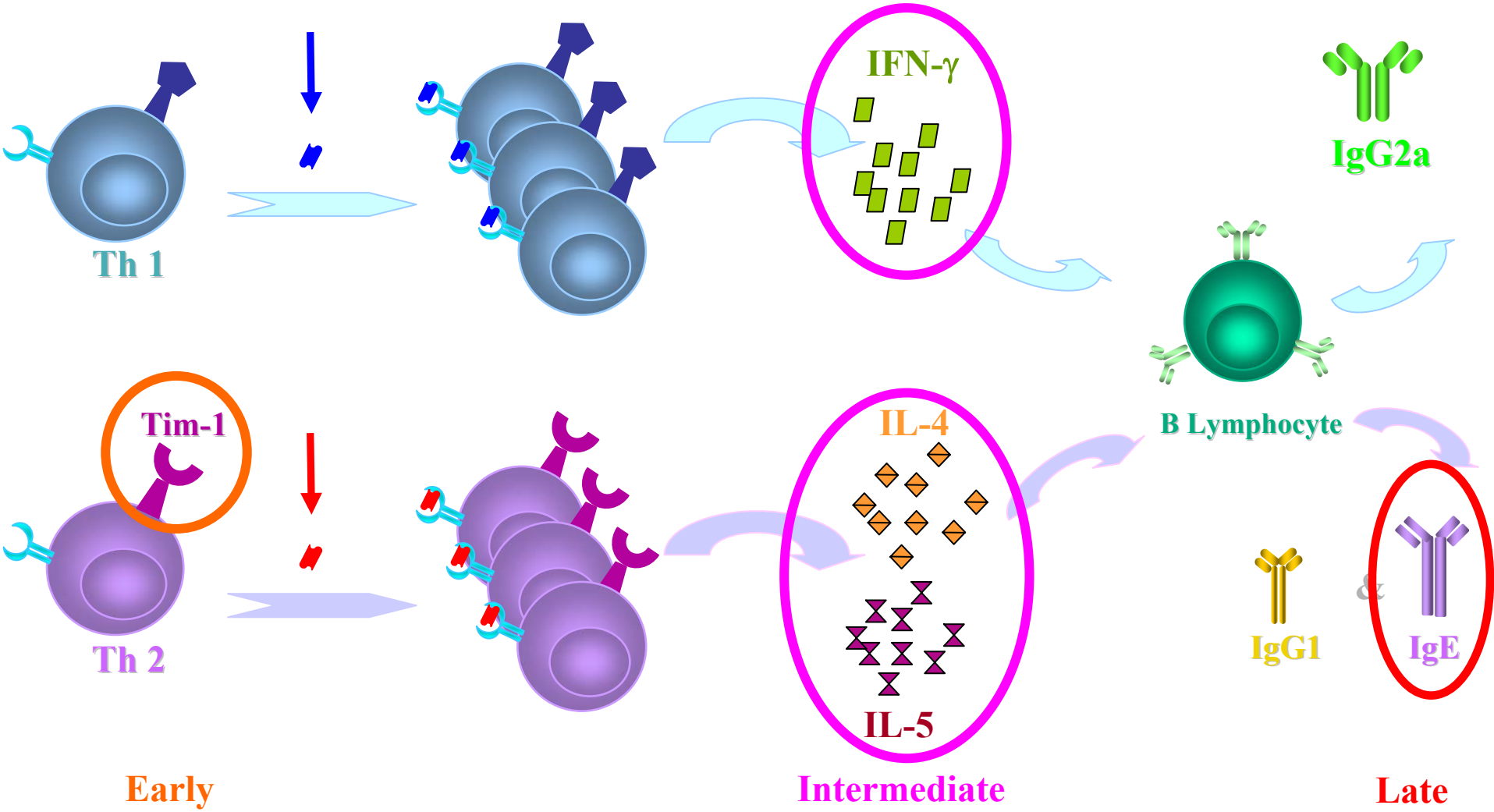


Different concentrations of Ara h 1, PAP or PNA were tested.

<u>Protein Concentrations</u>

0.01 g/l (0.0025 mg/mouse)
0.04 g/l (0.01 mg/mouse)
0.16 g/l (0.04 mg/mouse)
0.64 g/l (0.16 mg/mouse)
2.50 g/l (0.64 mg/mouse)

The parameters: Tim-1, IL-5/IFN-γ, IgE



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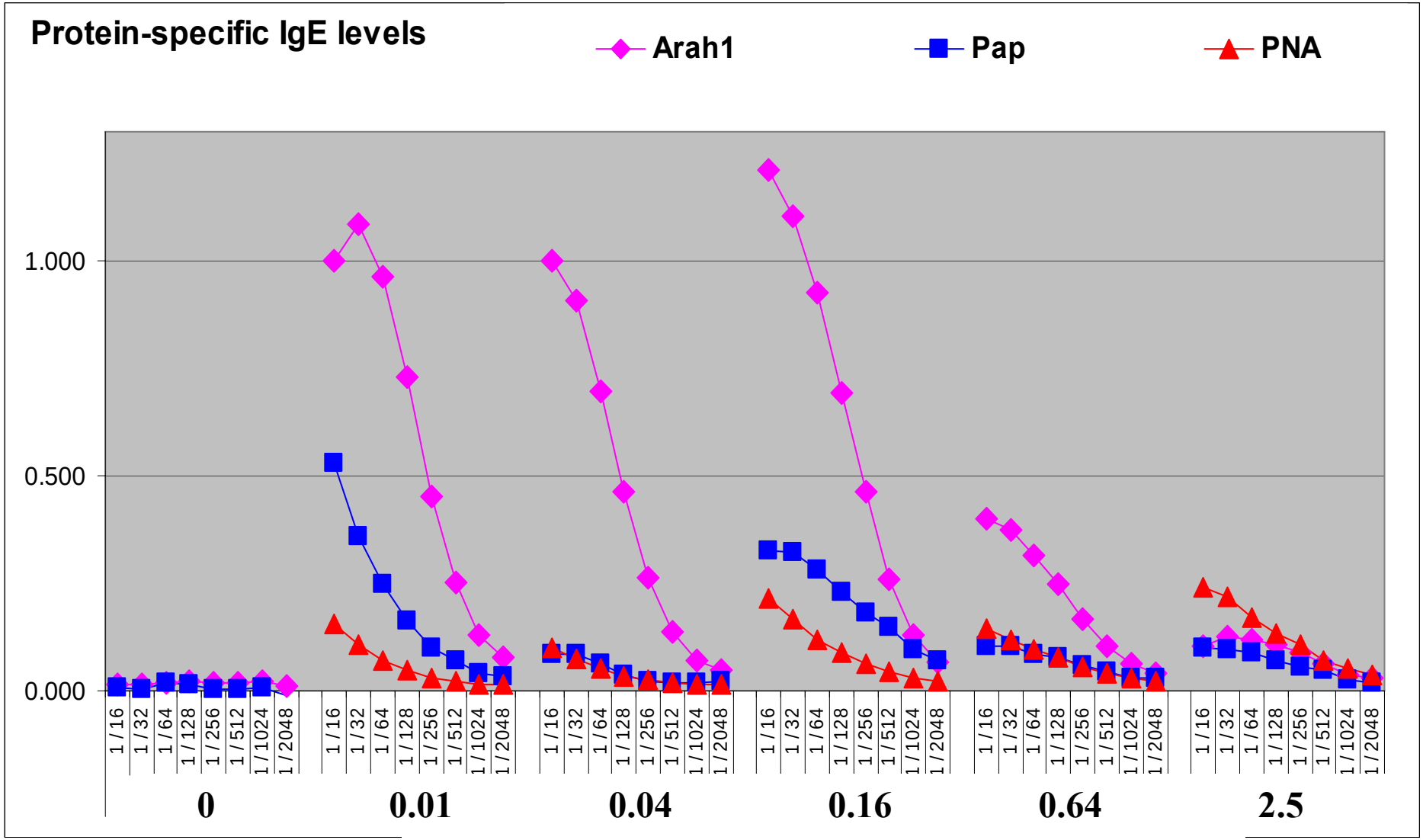
Late Marker => Protein-specific IgE (ELISA)



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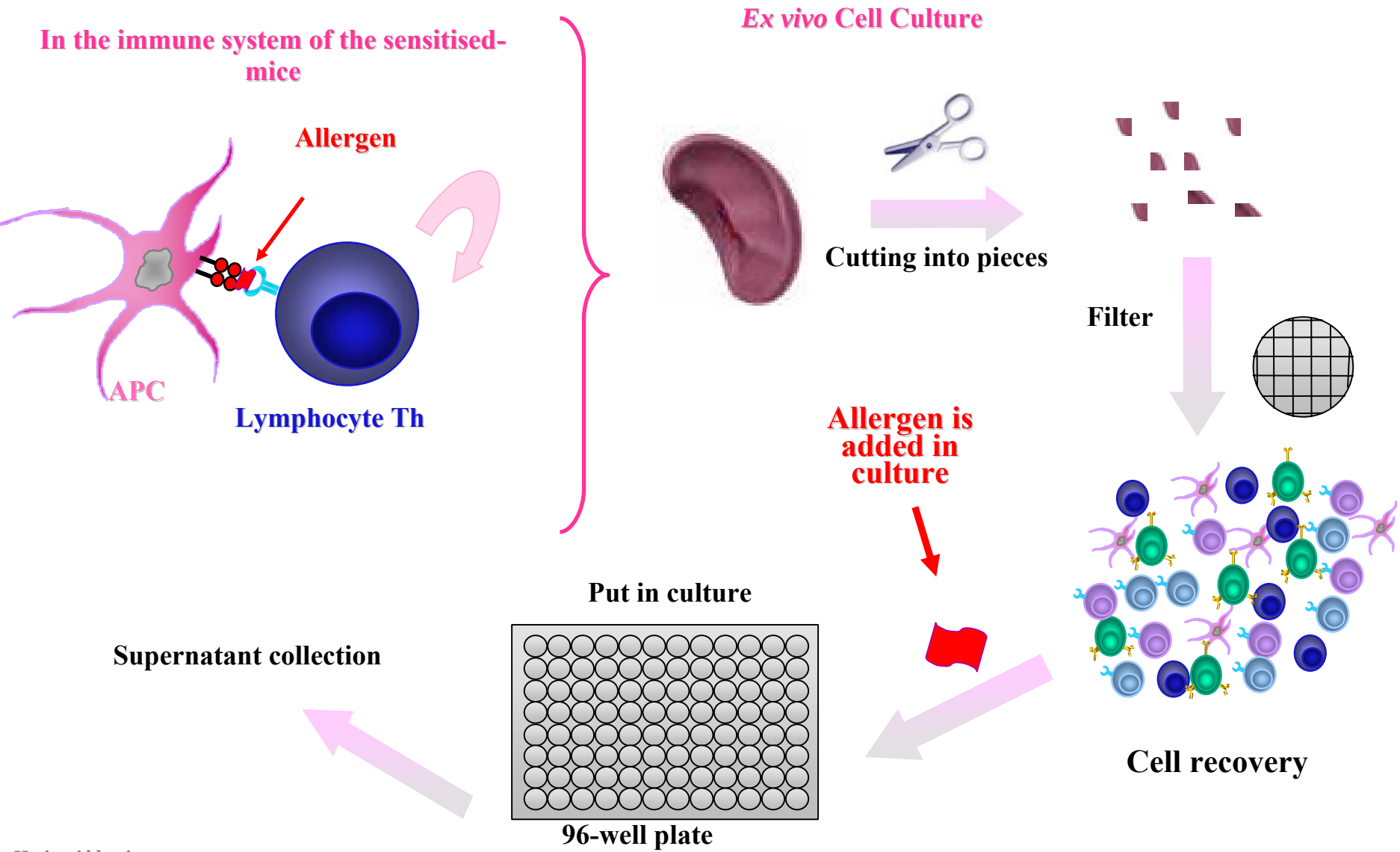
Splenocyte culture for cytokine expression



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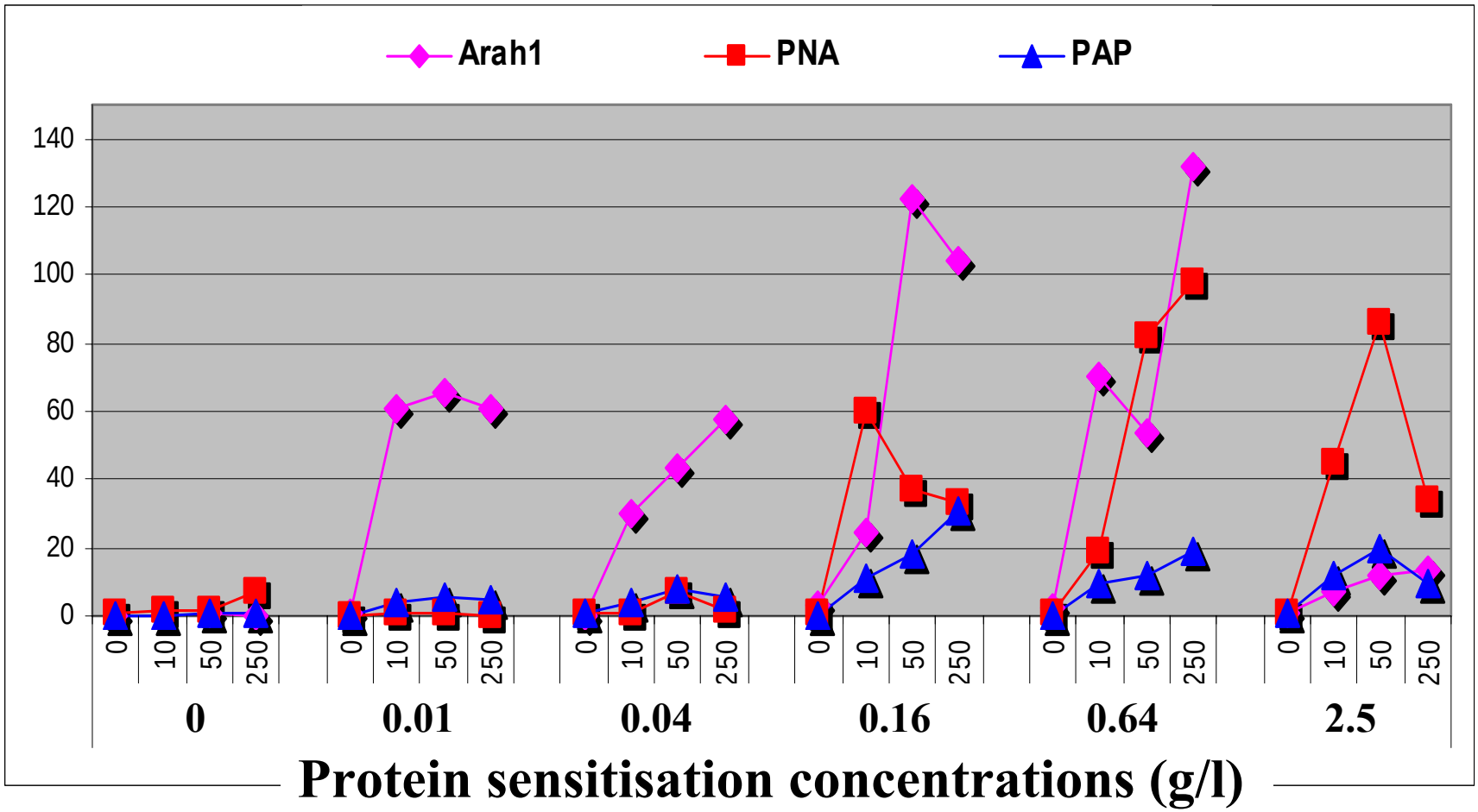
Intermediate Markers => IL-5 / IFN-γ Ratio



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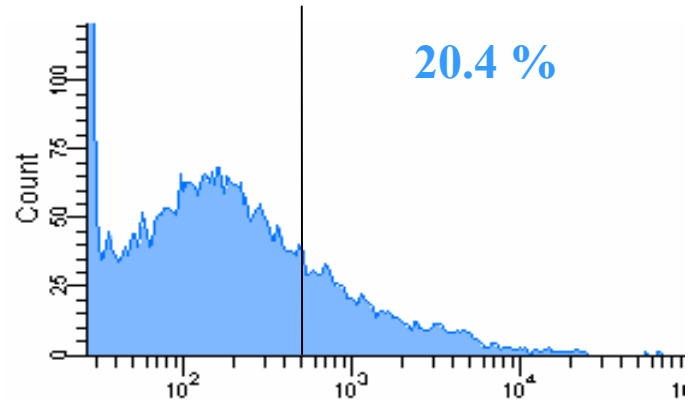
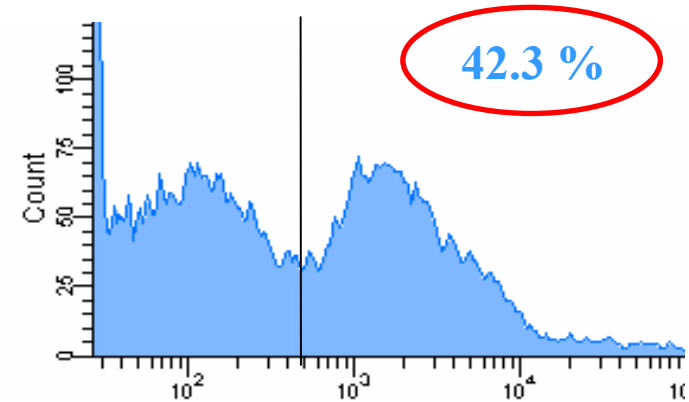
H. Aldemir, R. Bars, C. Herouet-Guicheney. Partly presented in ITCASS, Lodi, Italy, 2006.

Early marker => Surface Tim-1 expression

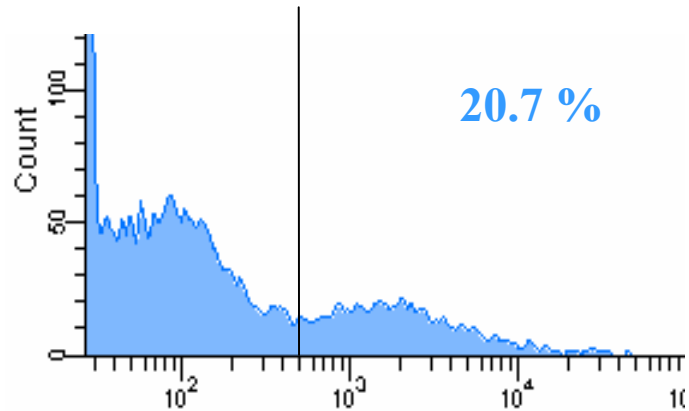
Control-Arah1



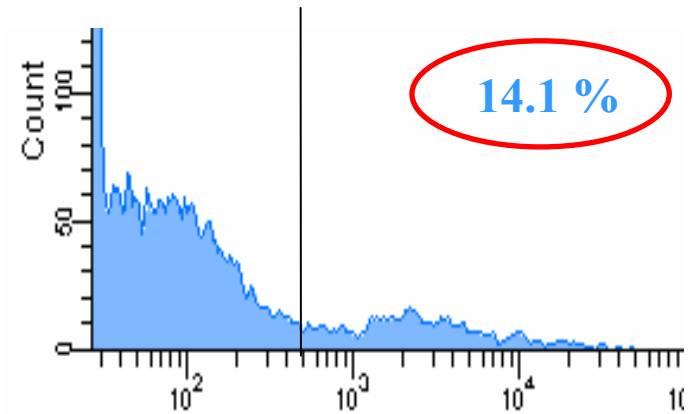
Allergen Ara h 1



Control-PAP



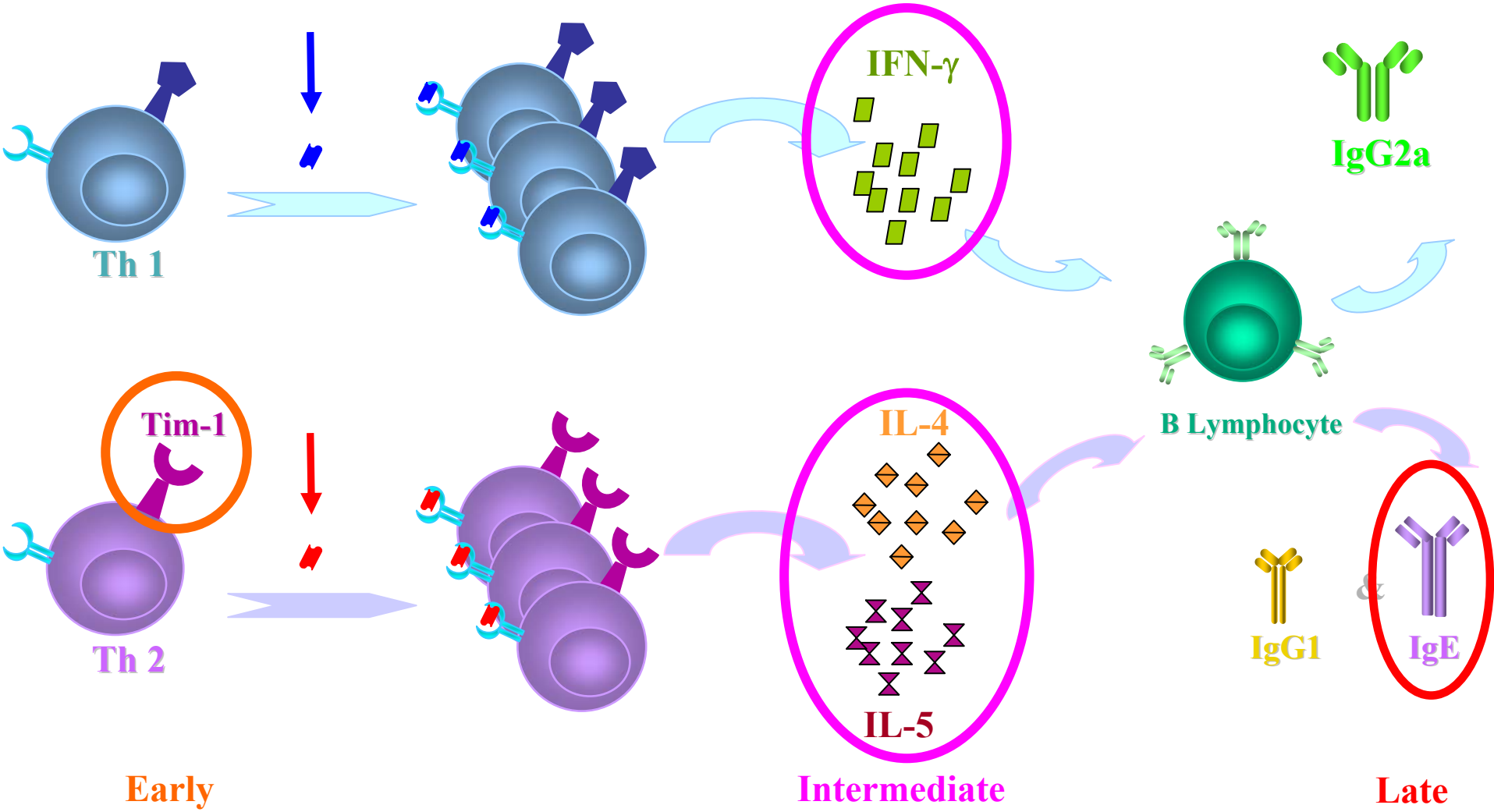
Non-Allergen PAP



Tim-1 surface expression

Hatice Aldemir

The parameters: Tim-1, IL-5 / IFN-γ, IgE



Hatice Aldemir

New model: Conclusion

This animal model with the selected parameters is promising to discriminate between

Strong Allergen **Weak allergen**

However: Standardisation of the model is necessary

General Conclusion

=> **Standardisation / International validation**

Need for more proteins to be tested

=> **Several animal models are needed**

* **Clinical model**	***vs.***	**Mechanistic model**
ASA scoring		**IgE, IgGs, cytokines, etc.**
* **Protein**	***vs.***	**Food**
Purity & conformation		**Matrix & Processing**

=> **Weigh of evidence approach (*Codex Alimentarius* 2003)**

Thank you very much

for your kind attention.

Sponsored by

Bayer CropScience

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Prof. N. Glaichenhaus (Univ. Nice)

BCS participants to the work

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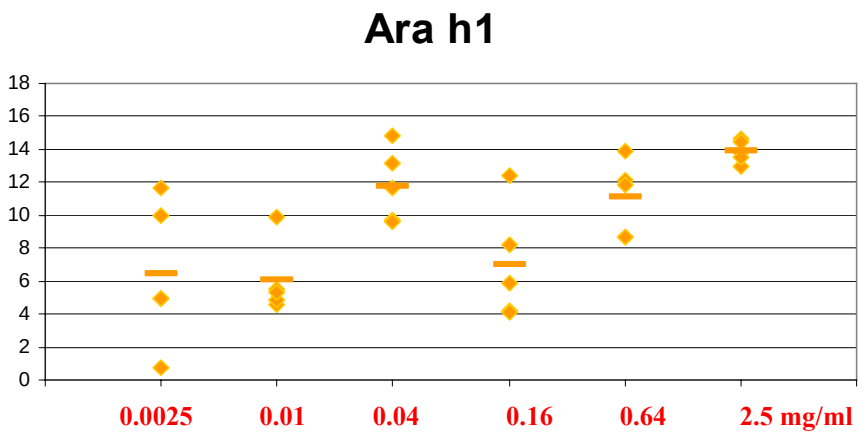
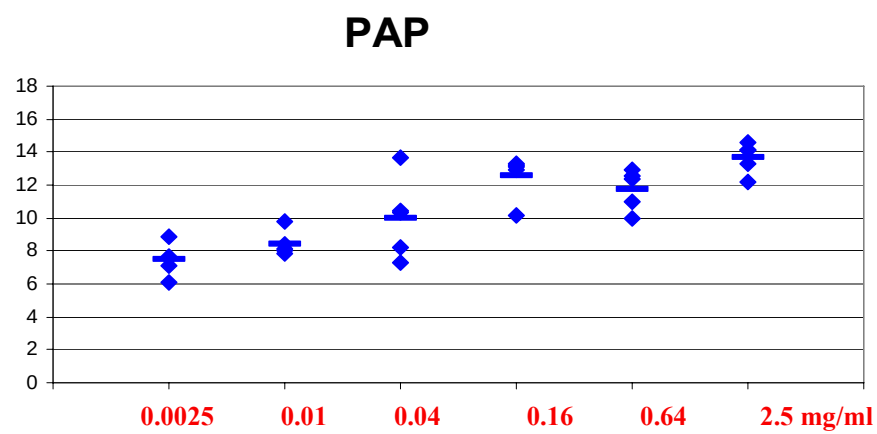
Dr. P. Kennel

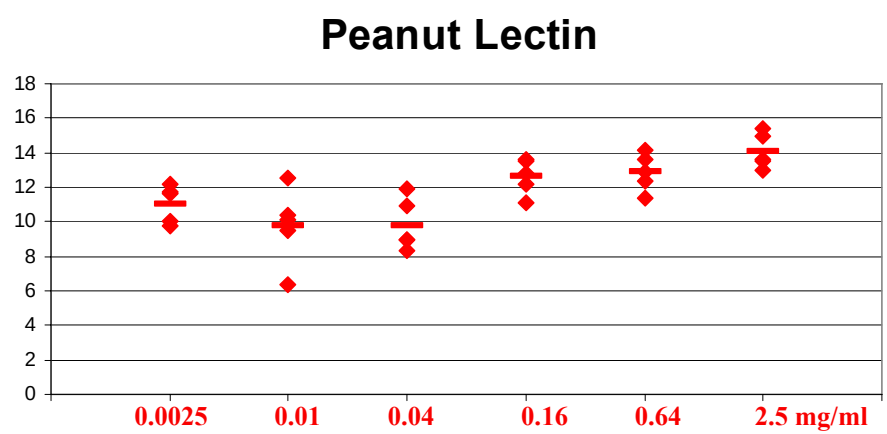
Dr. D. Rouquié

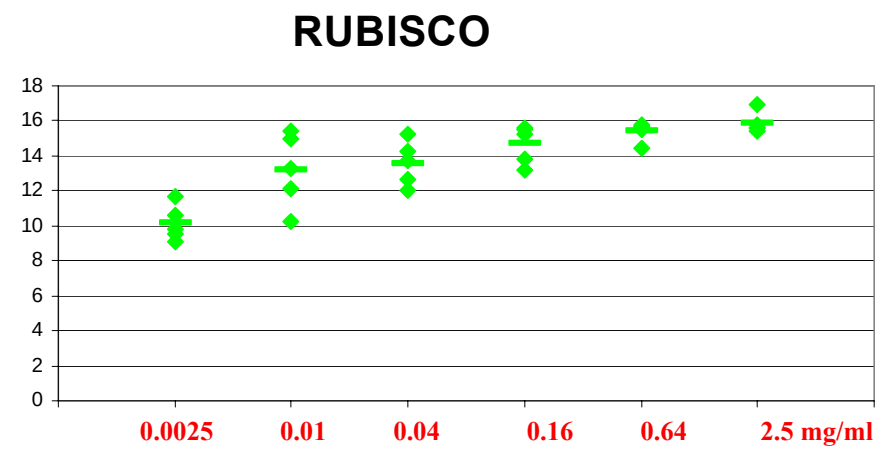
BCS Zootech & Technical Groups

Late Marker => Protein-specific IgG









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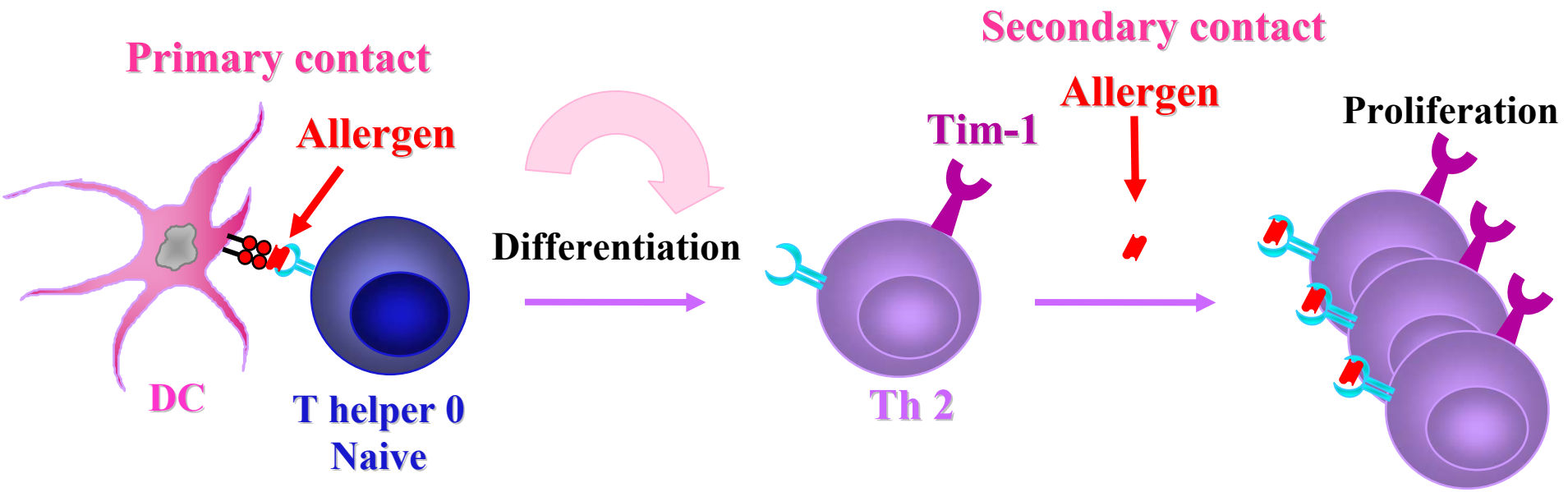
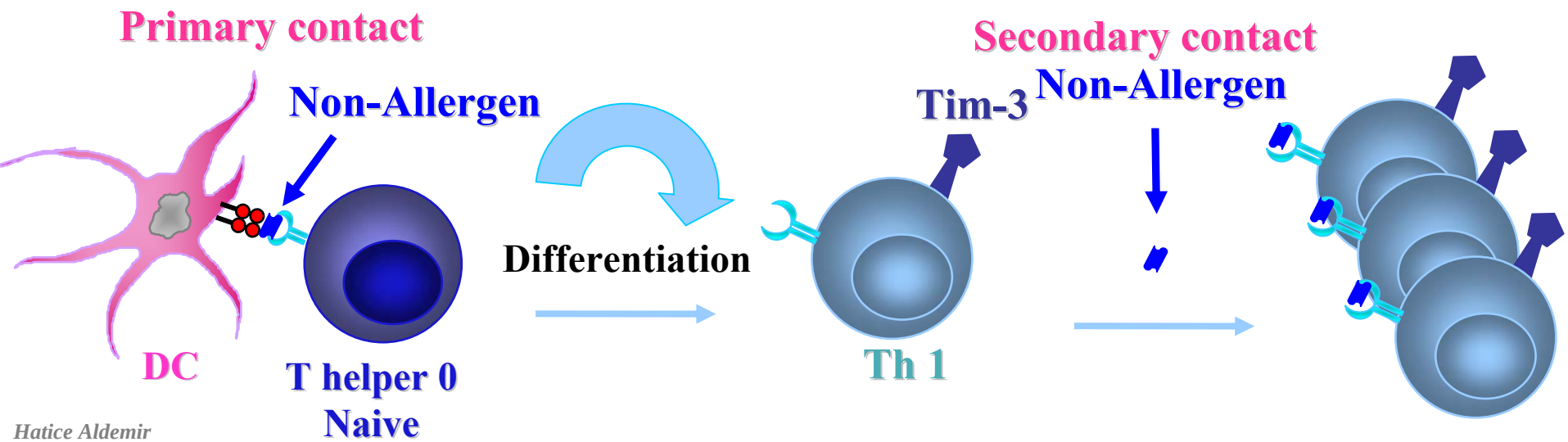
Molecular mechanism of allergy





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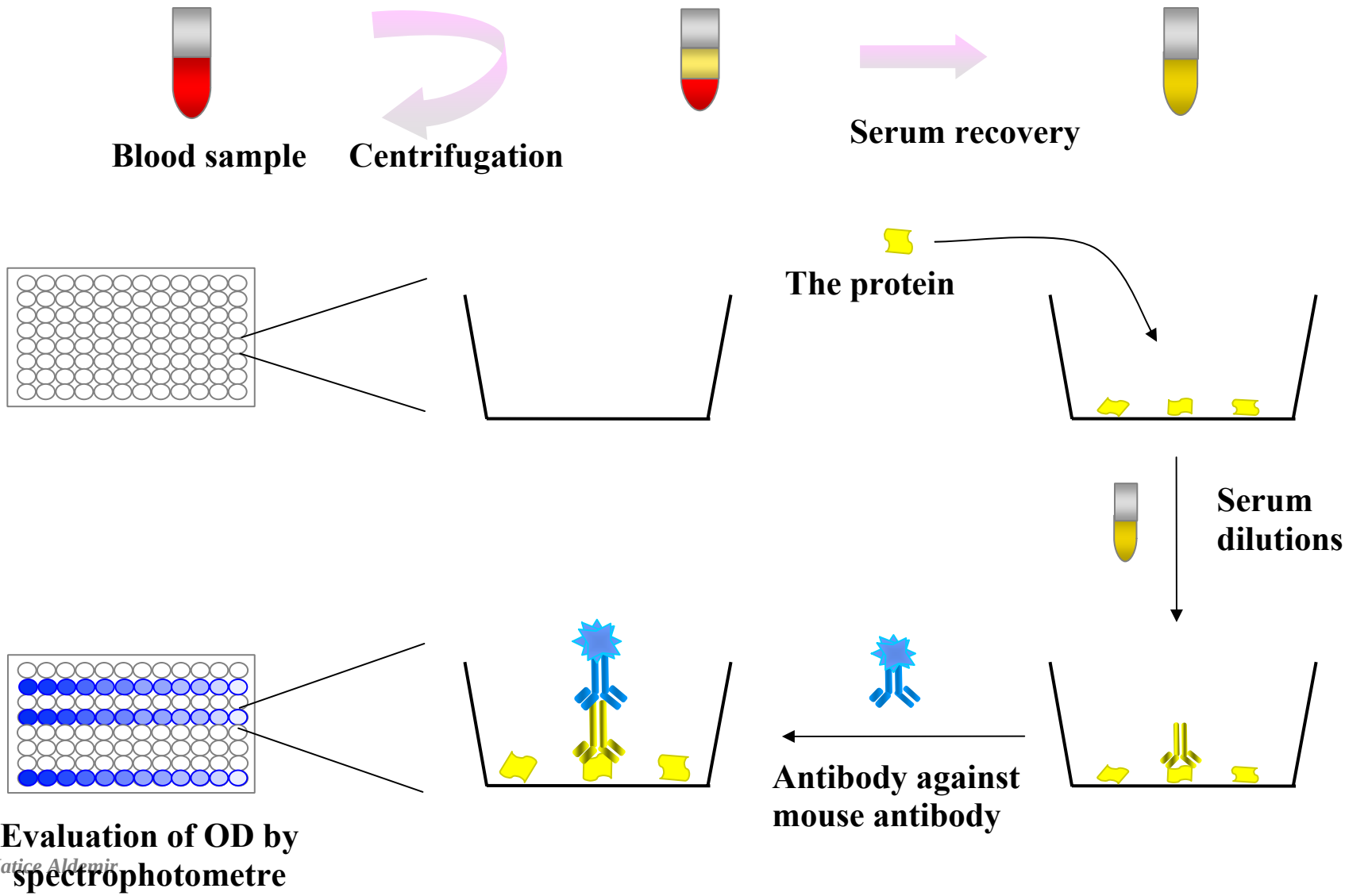
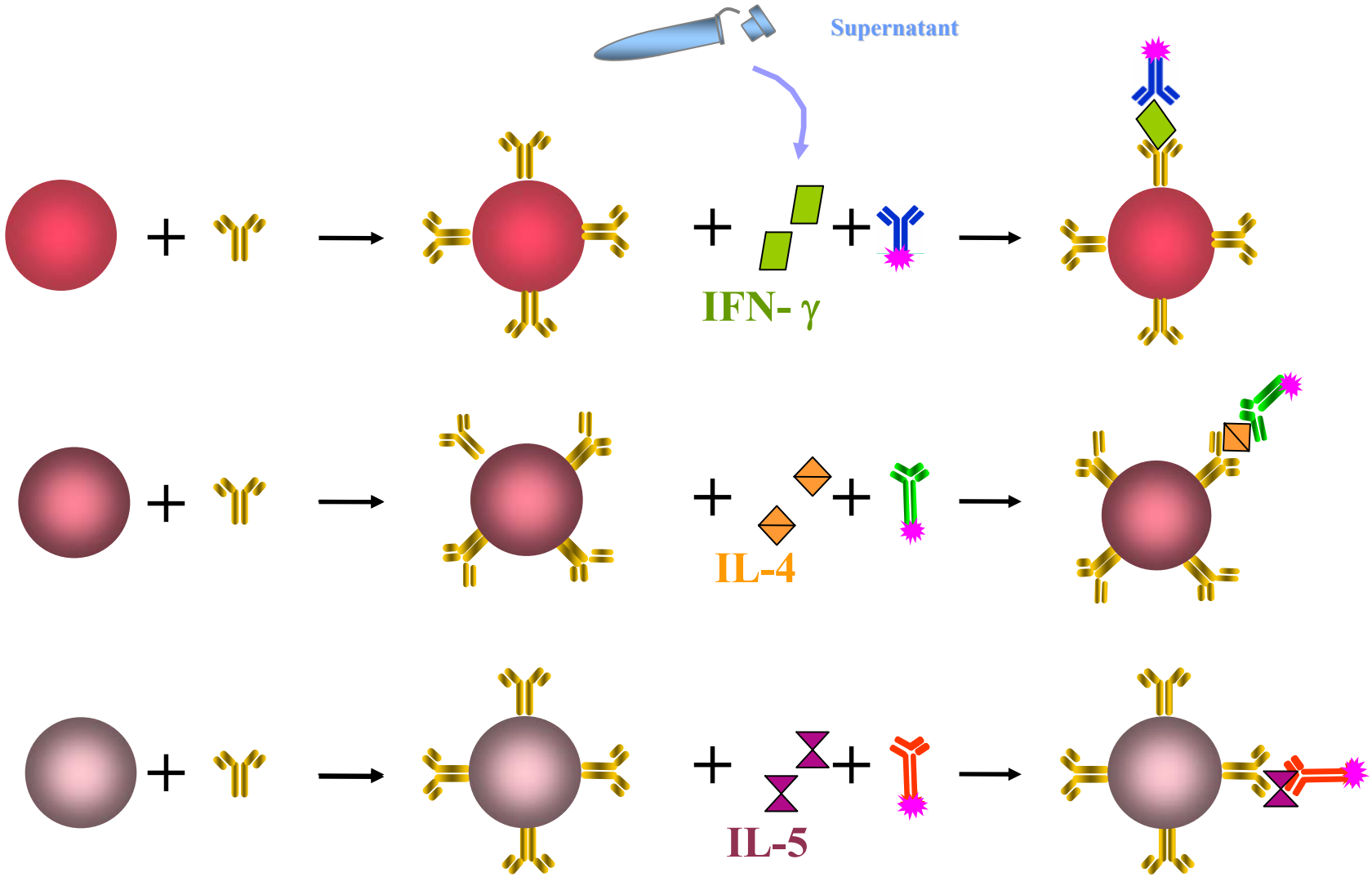
ELISA (Igs, Cytokines, etc..)



Cytometric Bead Array (CBA)



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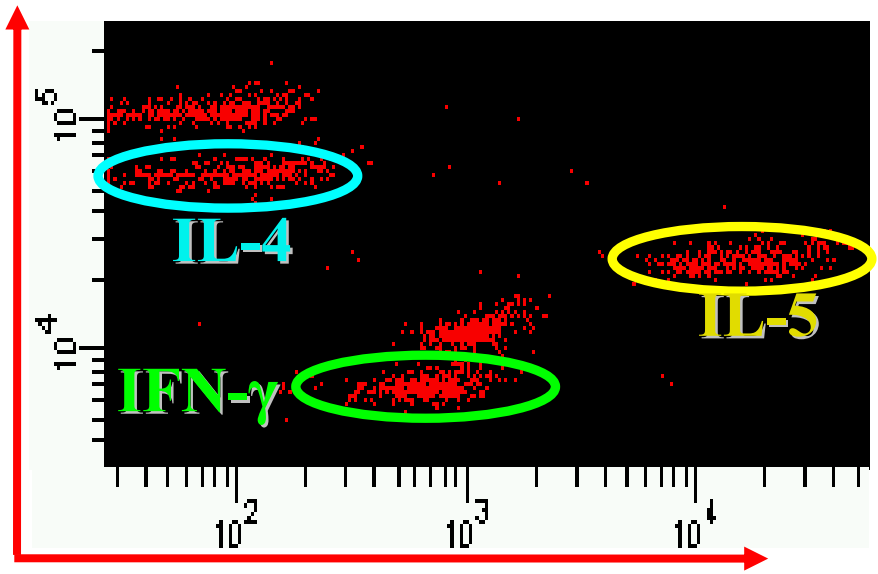
Analysis of cytokines by Cytometric Bead Array (CBA)

Color of the beads

=

Differentes cytokines



Fluorescence Intensity

=

Quantity of cytokine

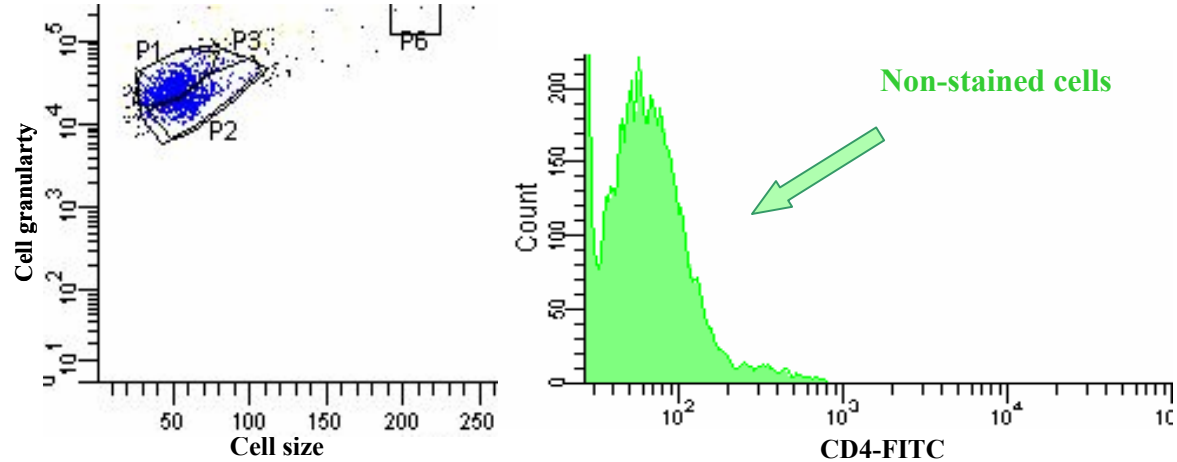
Becton Dickinson (BD)

FACS
In CNRS Lab.

CD4 => T helper cell marker

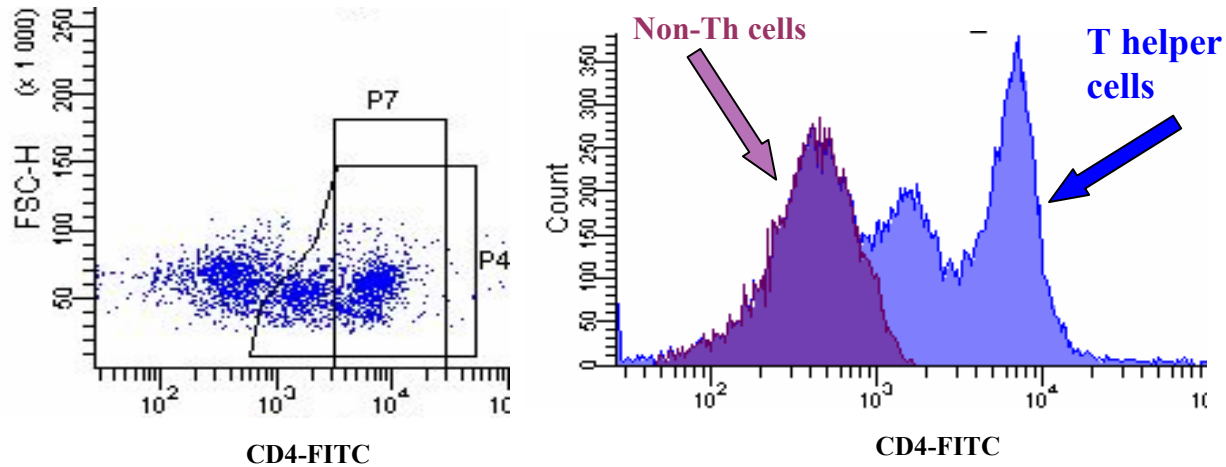
Total splenocytes

Total splenocytes are analysed according to size and granularity



T helper marker-stained cells

T helper cells are marked with the specific antibody CD4-FITC



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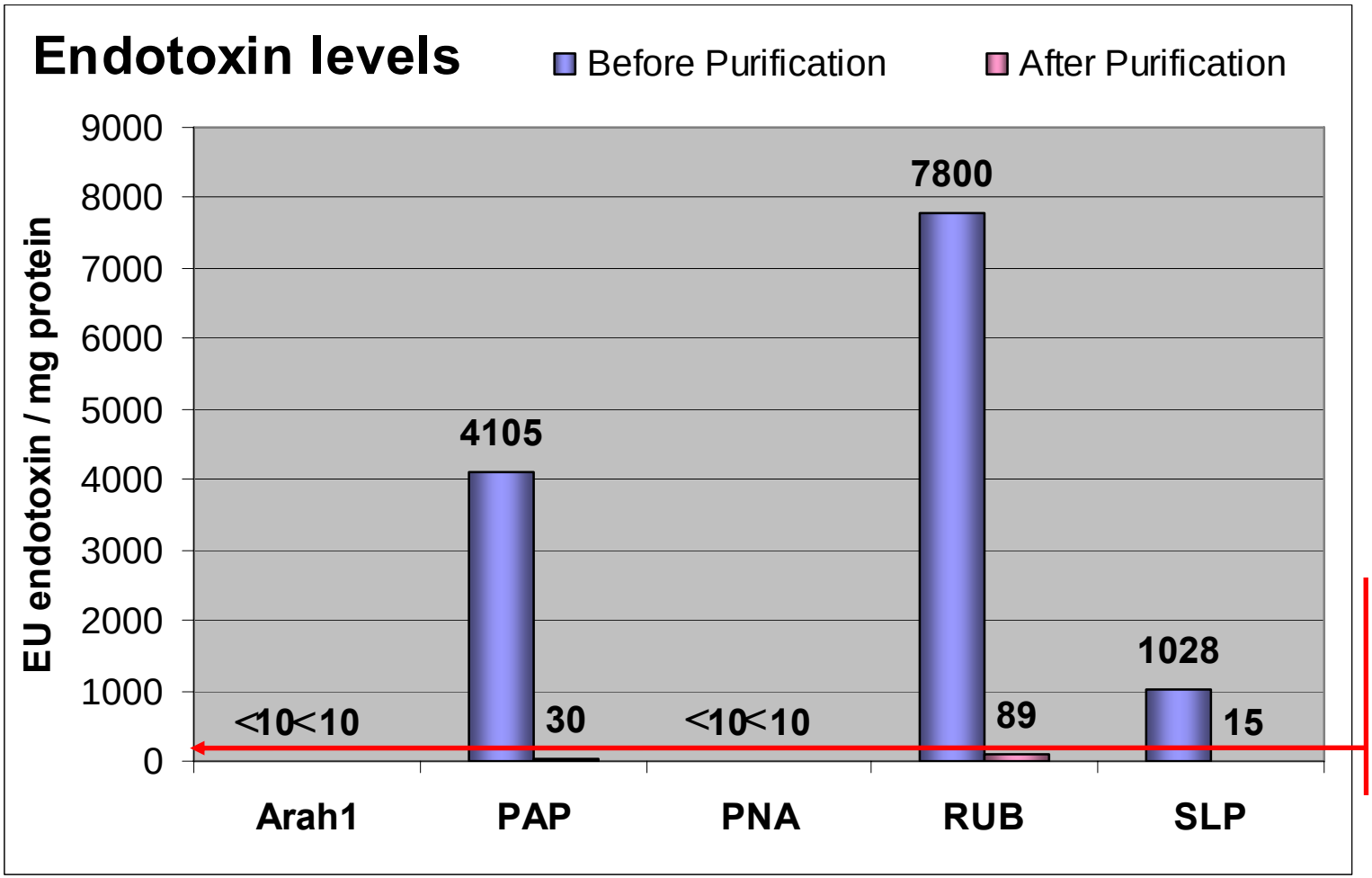
Endotoxins (Endotoxin Units / mg protein)



100 EU/mg

Threshold for cytokine response bias.

Ref: Dearman 2007