In vitro methods for predicting allergenicity

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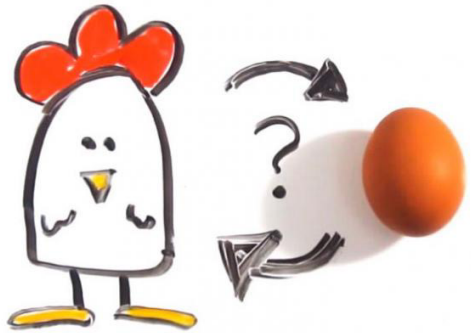
Icahn School of Medicine at Mount Sinai

Working Group 2: **In vitro *methods for predicting sensitization***

In vitro methods for predicting allergenicity

A chicken-and-egg situation:



What makes a food protein an allergen?
Does digestion of food proteins affect their allergenicity?

On the cutting edge of reactions triggered by food:

Availability, strengths, and limitations of in vitro methods for allergenic sensitization assessment.
What's about the celiac disease? And the GMOs?

In vitro methods for predicting allergenicity

Dr. Joana Costa
Protein stability

Dr. Alan Mackie
Protein digestion

Dr. Katrine Bogh
In vitro *approaches for allergenicity*

Dr. Frits Koning
Binding affinity in celiac disease

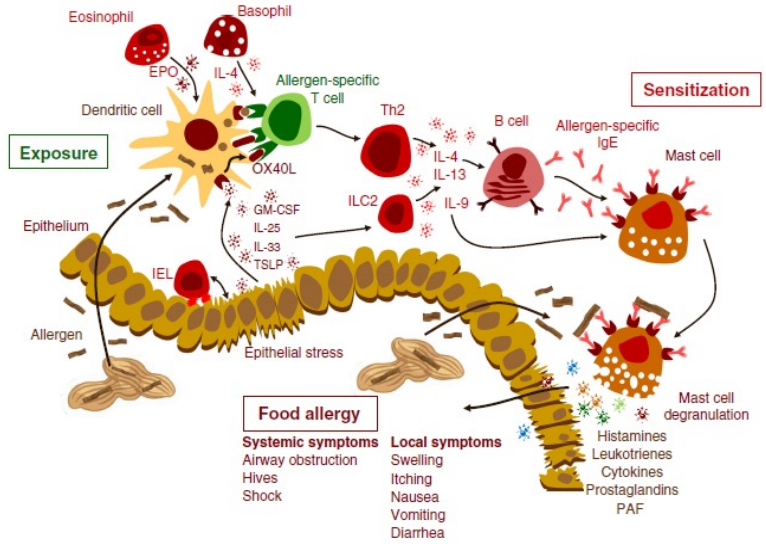
Dr. Andre Silvanovich
Allergenicity assessment of GMOs – CropLife Europe

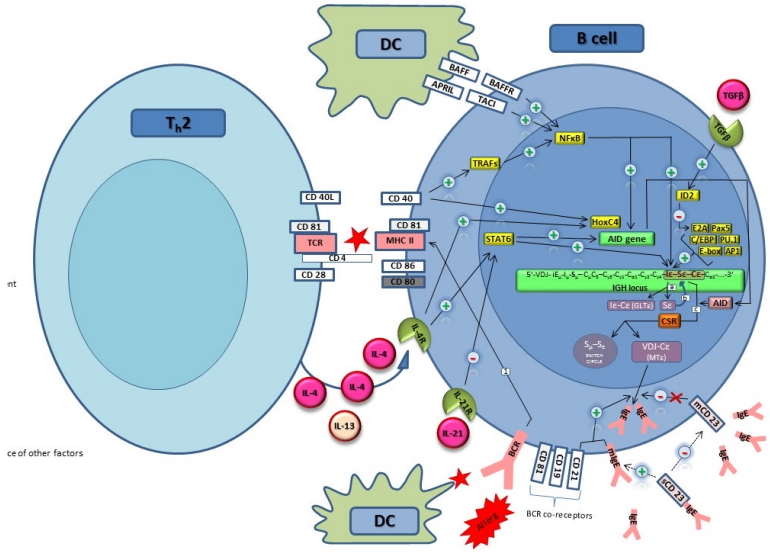
Novel protein-rich foods derived from plants and animals

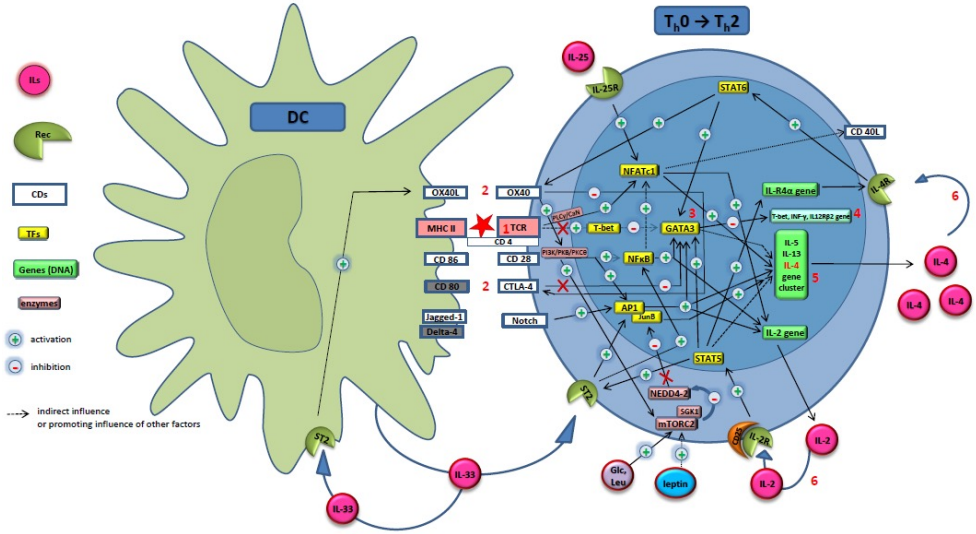
RISK ASSESSMENT: ALLERGENICITY

(Selb et al., 2017, Food Chem. Tox., 101, 139-148)

Biological events involved in sensitization to food proteins







Working Group for In Vitro

Methods to Predict Sensitization

(Adapted from Smit et al., 2015, Drug Discov. Today, 17-19, 63-69.Van Bilsen et al., 2017, Clin. Transl. Allergy, 7, 18)

Adverse Outcome Pathway (AOP) concept

ImpARAS

Working Group for In Vitro

Methods to Predict Sensitization

van Bilsen *et al. Clin Transl Allergy (2017) 7:13*
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Clinical and Translational Allergy

REVIEW Open Access

Application of the adverse outcome pathway (AOP) concept to structure the available in vivo and in vitro mechanistic data for allergic sensitization to food proteins

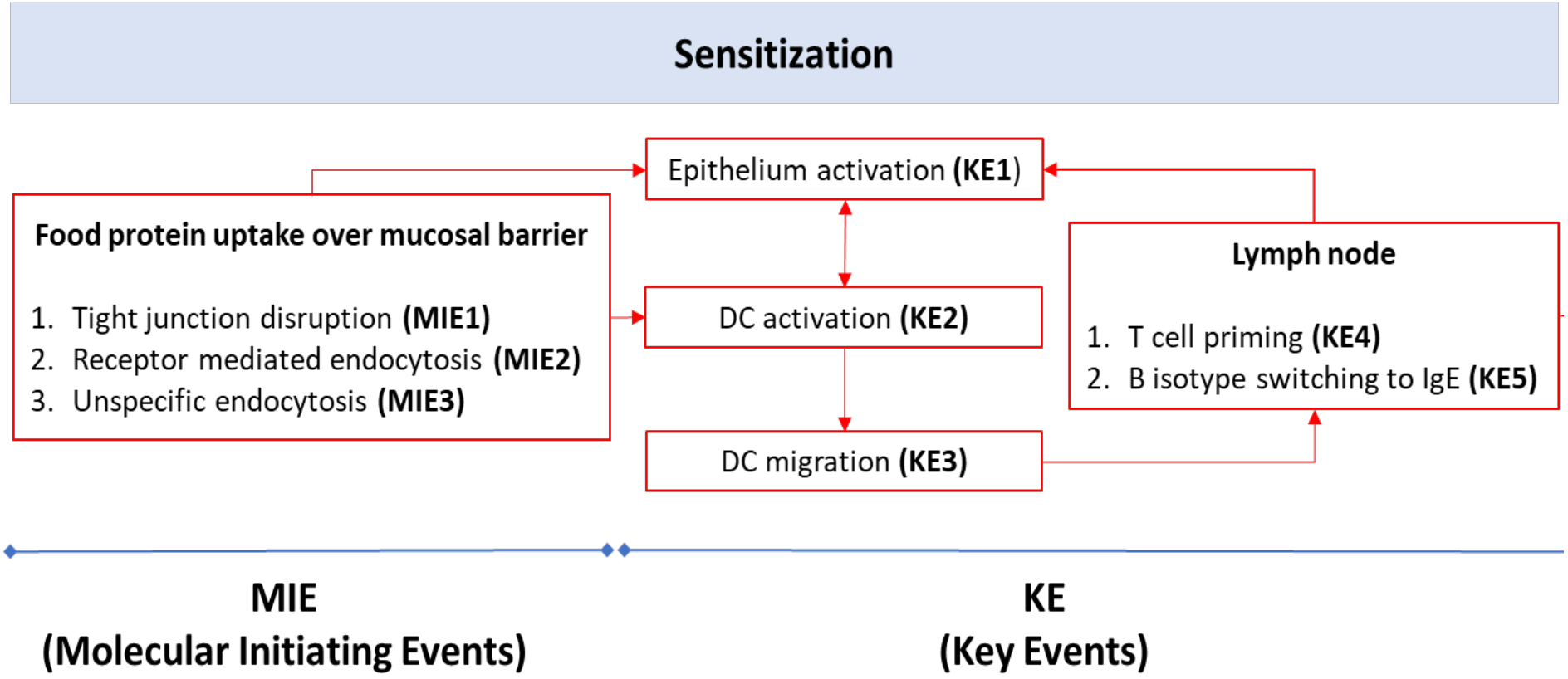
Jolanda H. M. van Bilsen[1*], Edyta Sienkiewicz-Szłapka[2], Daniel Lozano-Ojalvo[3], Linette E. M. Willemsen[4], Celia M. Antunes[5], Elena Molina[3], Joost J. Smit[4], Barbara Wróblewska[6], Harry J. Wichers[7], Edward F. Knol[8], Gregory S. Ladics[9], Raymond H. H. Pieters[4], Sandra Denery-Papini[10], Yvonne M. Vissers[11], Simona L. Bavaro[12], Colette Larré[10], Kitty C. M. Verhoeckx[1] and Erwin L. Roggen[13]

What is an Adverse Outcome Pathway (AOP)?

- An adverse outcome pathway (AOP) is a structured representation of biological events leading to adverse effects.
- A stepwise approach of events (one event leads to another event)
- It helps us to understand the mechanisms of adverse effects at a biological level of organization

(Van Bilsen et al., 2017, Clin. Transl. Allergy, 7, 18)

Applying AOP to biological events involved in sensitization to food proteins



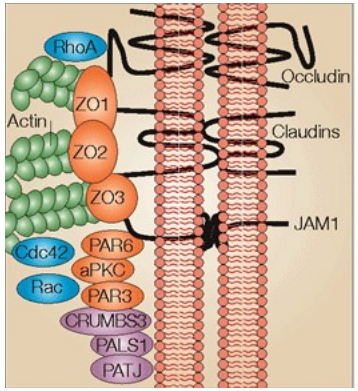
(Adapted from Van Bilsen et al., 2017, Clin. Transl. Allergy, 7, 18)

OBJECTIVES

We aimed to provide the state-of-the-art of existing *in vitro* approaches for assessing sensitizing potential of food proteins based on the identified MIE and KE proposed in the AOP for allergic sensitization to food proteins.

For this propose, we clustered and structured the existing *in vitro* models that are suitable to study the major events involved in allergic sensitization, focusing on major read-outs as well as strengths and limitations of these assays.

In vitro models to assess tight junction disruption (MIE1)



In vitro models:

- Caco-2
- HT-29
- T84
- IPEC-J2

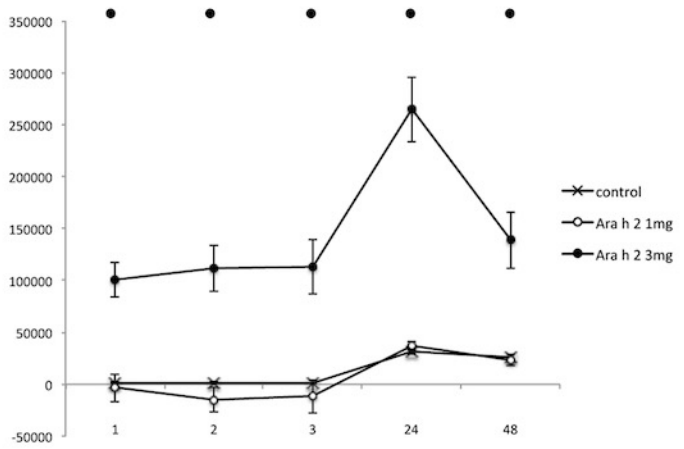
Disruption evaluation:

- Transepithelial Electrical Resistance (TEER)
- Blue Dextran leakage assay
- Lucifer Yellow assay

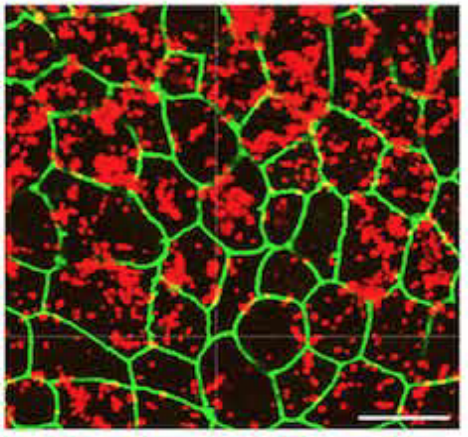
Cleavage of tight junctions:

- Western blotting
- Confocal microscopy
- RT-qPCR
- Mass spectrometry (tight junctions proteolysis)

- **Kiwifruit** (Act d 1)
- **Pineapple** (Ana c 2)
- **Papaya** (Car p 1)
- **Peanut** (Ara h 2)
- **Gluten** (Gliadins)



Transport of Ara h 2



Modifications of Occludin, JAM-A, claudin-1, and ZO-1

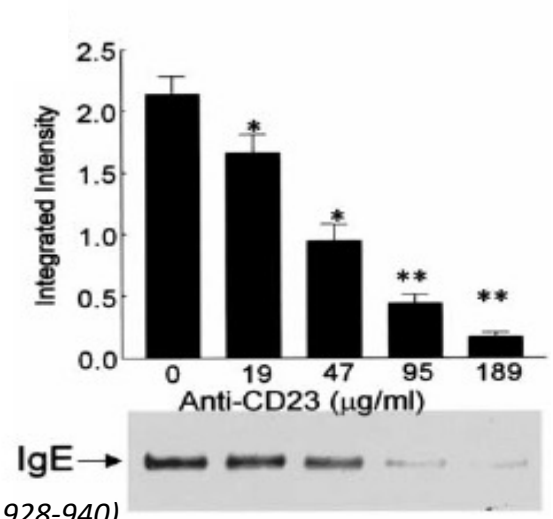
(Price et al., 2014, Cell Physiol. Biochem., 33, 1758-1777)

In vitro models to assess receptor-mediated (MIE2) and unspecific endocytosis (MIE3)

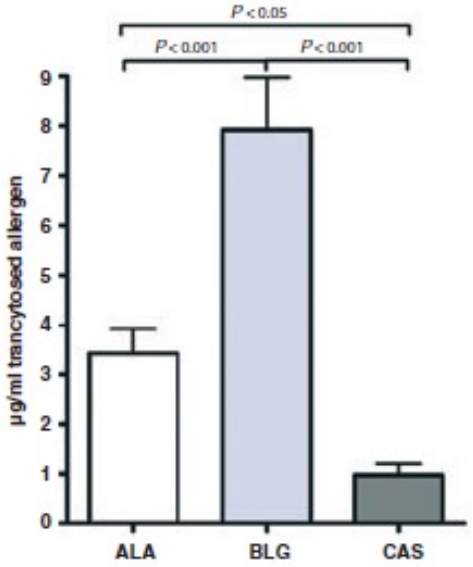
MIE2: The role of CD23

In vitro models:

- Caco-2
- HT-29
- T84



(Tu et al., 2005, Gastroentorology, 129, 928-940)



(Roth-Walter et al., 2008, Allergy, 63, 882–890)

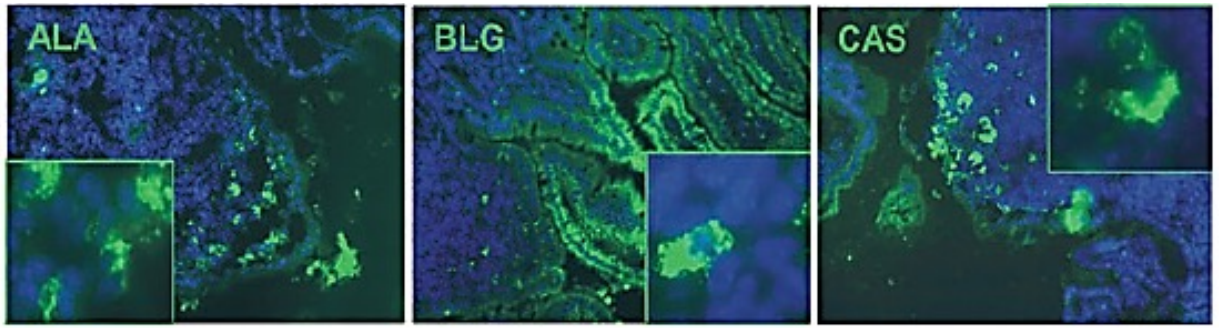
MIE3: Unspecific endocytosis

In vitro models:

- Caco-2
- HT-29
- Murine M cells

Quantification of allergen passage:

- ^{14}C-radiolabelled allergens
- Fluorophore-labelled allergens
- ELISA, SDS-PAGE and Western blotting



Transport mediated by endocytosis

In vitro models to assess epithelium activation (KE1)

In vitro models:

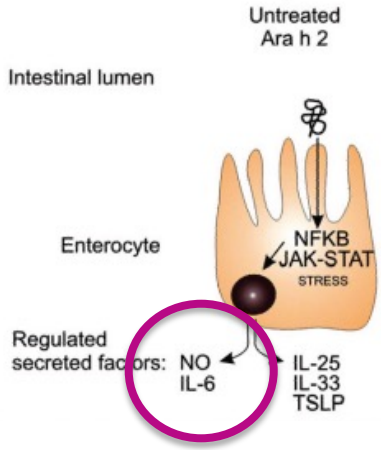
- Caco-2
- HT-29
- T84

Evaluation of the epithelial activation:

- Intracellular ROS generation
- Cytokine production
- Mucus secretion
- Ca^{2+}-signaling pathways

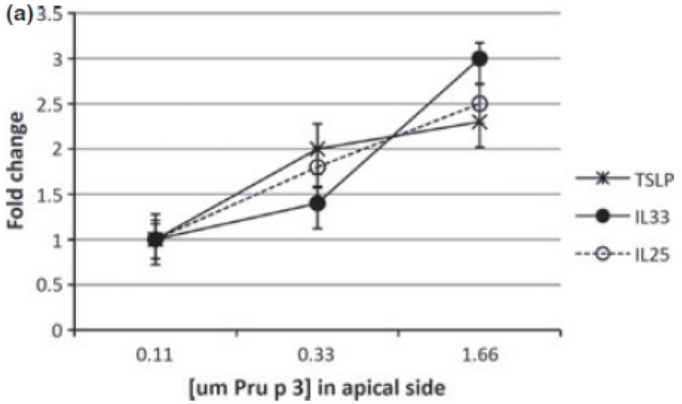
- Cow's Milk proteins (α-lactalbumin and β-lactoglobulin)
- Peanut (Ara h 2)
- Egg (ovomucoid and ovalbumin)
- Peach (Pru p 3)
- Wheat proteins (β-, γ-, ω1,2-, ω5-gliadins)
- Soybean (P34)
- Brazil nuts (Ber e 1)
- Sesame seeds (Ses i 1)

Intracellular ROS Generation



(Starkl et al., 2011, Open Allergy J., 4, 24-34)

Cytokine production



(Tordesillas et al., 2013, Clin. Exp. Allergy, 43, 1374-1383)

In vitro models to assess dendritic cell activation and migration (KE2 and KE3)

In vitro models:

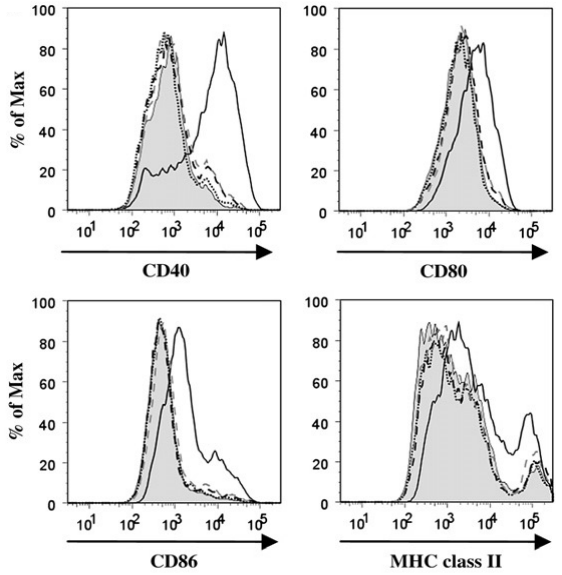
- THP-1-derived DCs
- Human monocyte-derived DCs (moDCs)
- Murine bone marrow-derived DCs (BM-DCs)

Evaluation of the DC activation:

- Surface markers
- Cytokine production
- Allergen uptake

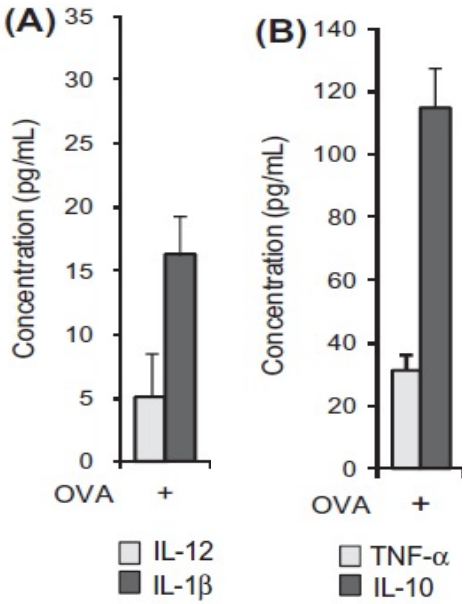
- Cow's Milk proteins (Bos d 5)
- Peanut (Agglutinin)
- Egg (ovalbumin)
- Peach (Pru p 3)
- Hazelnut (Cor a 8)
- Carrot (Dau c 1)
- Celery (Api g 1)
- Apple (Mad d 1)

DC activation



Maturation markers: CD40, CD80, and MHC-II

(Ilchmann et al., 2010, JACI, 125, 175-183)

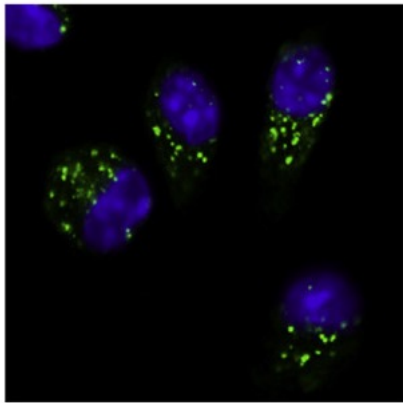


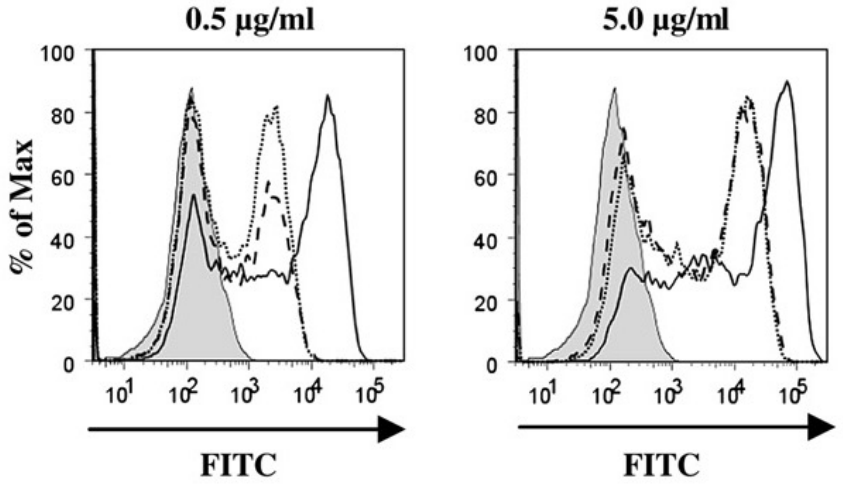
Increased production of IL-1β and IL-10

(Katayama et al., 2013, Food Chem., 138, 757-761)

In vitro models to assess dendritic cell activation and migration (KE2 and KE3)

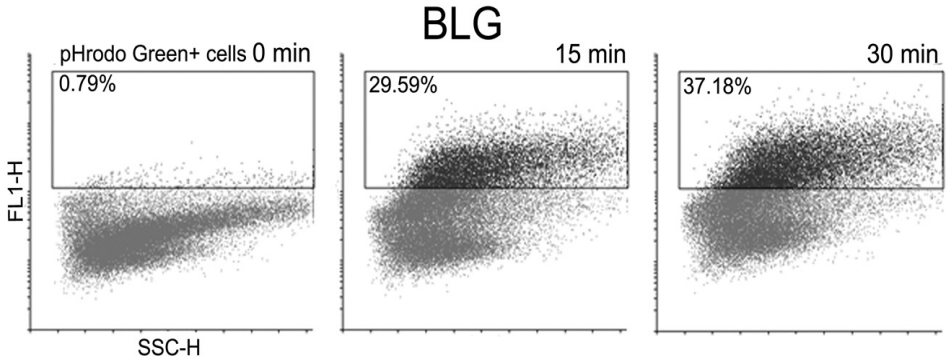
Allergen uptake

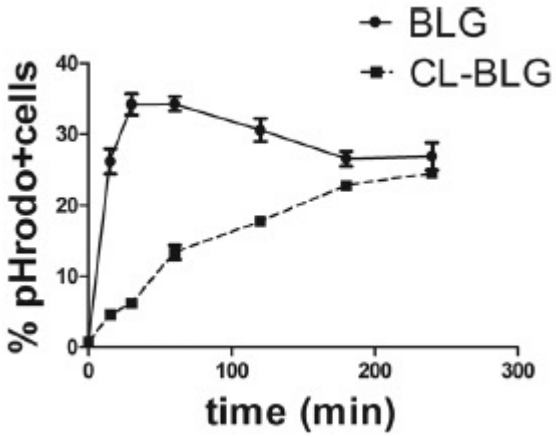




Enhanced uptake of glycosylated OVA (BM-DCs)

(Ilchmann et al., 2010, JACI, 125, 175-83)





Enhanced uptake of intact β-LG (BM-DCs)

(Stojadinovic et al., 2014, Toxicol. Sci., 140, 224-35)

In vitro models to assess Th2 cell priming (KE4)

Human *in vitro* models employed:

- Isolated PBMCs
- T cell lines
- T cell clones

Evaluation of T cell activation :

- Proliferation
- Surface marker expression
- Cytokine secretion

Murine in vitro models employed:

- Isolated cells from spleen, MLN, and lamina propria
- Primed CD4+ T cells from sensitized mice
- CD4+ T cells from TCR trangenic mice (DO11.10 and OTII)

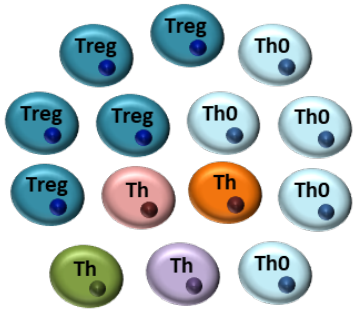
- **Cow's Milk proteins**
- **(casein, α-lactalbumin and β-lactoglobulin)**
- **Peanut**
- **(Ara h 1, Ara h 2, Ara h 3 and Ara h 6)**
- **Egg (ovalbumin and ovomucoid)**
- **Peach (Pru p 3)**
- **Hazelnut (Cor a 8)**
- **Apple (Mal d 1)**
- **Walnut (Jug r 1, Jug r 2 and Jug r 3)**
- **Tree nuts (Ana o 1 and Ana o 2)**

Challenge: To identify and characterize allergen-specific T cells

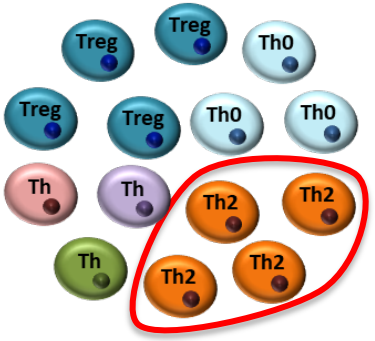
Detection of allergen-specific T cells

APPROACHES FOR THE STUDY OF ALLERGEN-SPECIFIC T CELLS

TOLERANCE



ALLERGY



Allergen-specific T cells, a very rare population

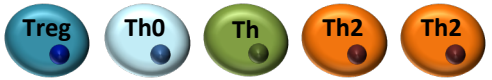
In vitro expansion

Intracellular cytokines

pMHC-II multimers

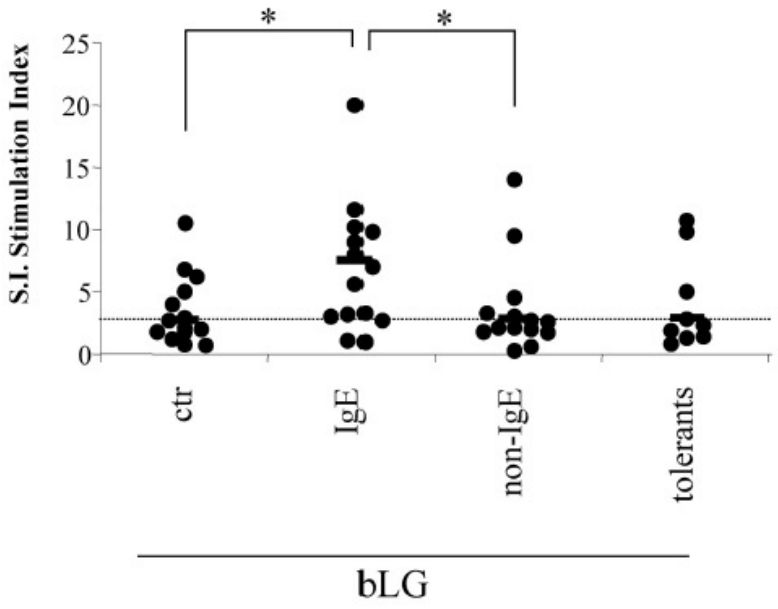
Activation markers

Identification



In vitro models to assess Th2 cell priming (KE4)

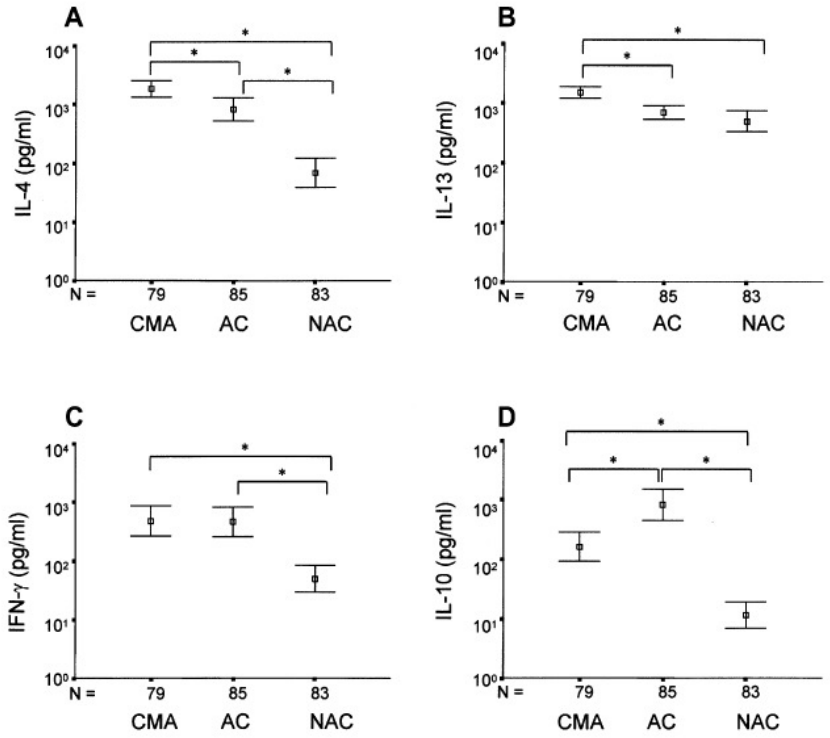
T cell proliferation



β-LG induces proliferation in PBMCs from CMA allergic patients

(Vocca et al., 2011, Pediatric Res., 70, 549–554)

Cytokine production



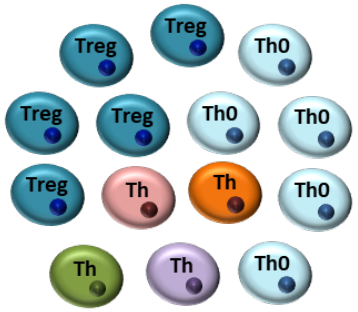
Increased production of IL4, IL13 and IFN-γ in T cell clones from CMA patients

(Tiemessen et al., 2004, JACI., 113, 932-939)

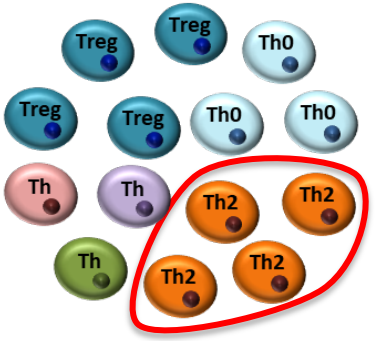
Detection of allergen-specific T cells

APPROACHES FOR THE STUDY OF ALLERGEN-SPECIFIC T CELLS

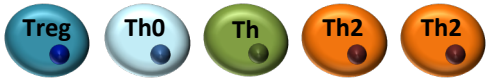
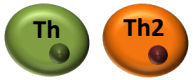
TOLERANCE



ALLERGY

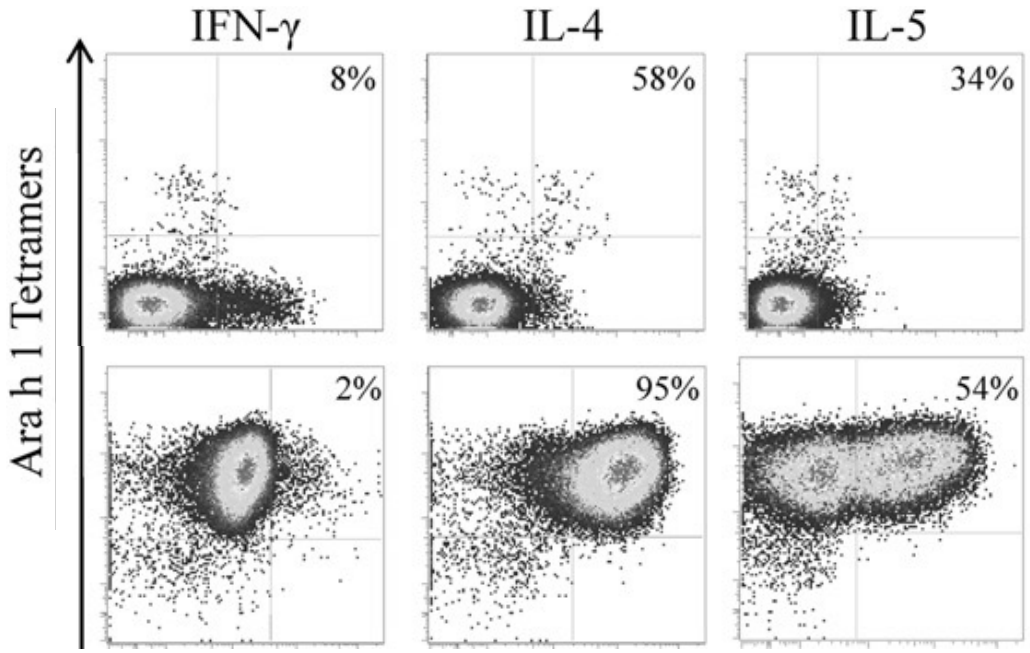


Allergen-specific T cells, a very rare population

	Identification	Limitation
***In vitro* expansion**		*Bystander activation* *Modifies % and phenotype*
Intracellular cytokines		
pMHC-II multimers		
Activation markers		

In vitro models to assess Th2 cell priming (KE4)

Intracellular Cytokine Production



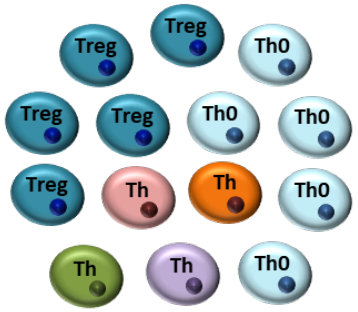
Increased production of IL4 and IL5 T cell clones from peanut patients

(DeLong et al., 2011, JACI, 127, 1211-1218)

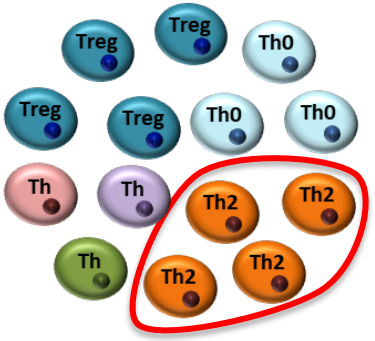
Detection of allergen-specific T cells

APPROACHES FOR THE STUDY OF ALLERGEN-SPECIFIC T CELLS

TOLERANCE



ALLERGY



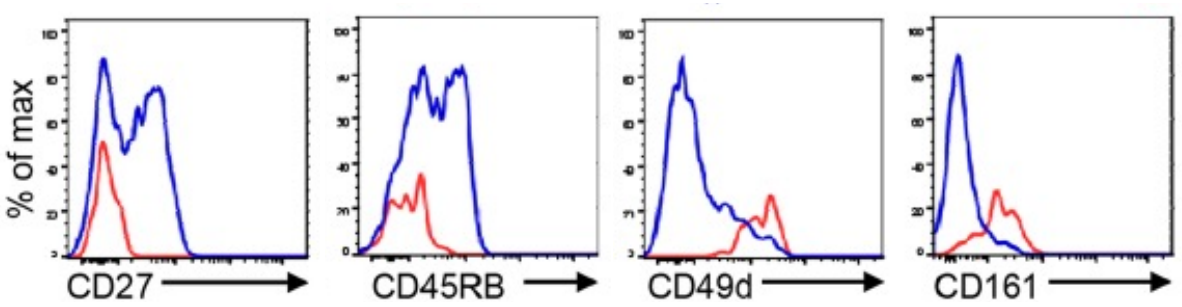
Allergen-specific T cells, a very rare population

	Identification	Limitation
***In vitro* expansion**	Treg Th0 Th Th2 Th2	*Bystander activation* *Modifies % and phenotype*
Intracellular cytokines	Th Th2	*Non-cytokine-producing* *Naive T cells and Treg*
pMHC-II multimers	Treg Th0 Th Th2	
Activation markers		

Identification of peanut-specific Th2 cells by pMHC-II TETRAMERS

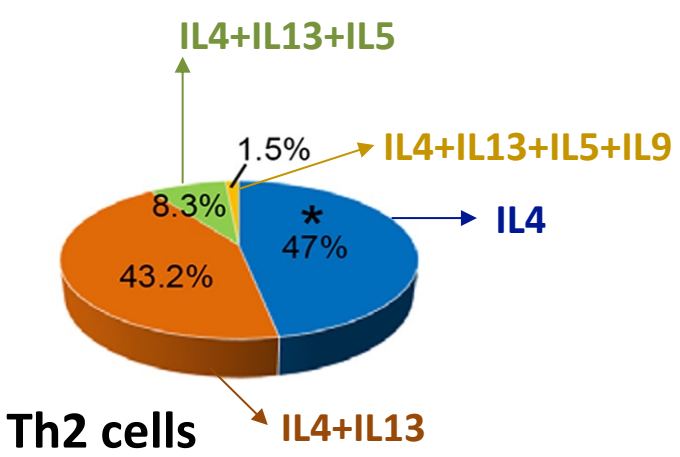
Total Th2 cells

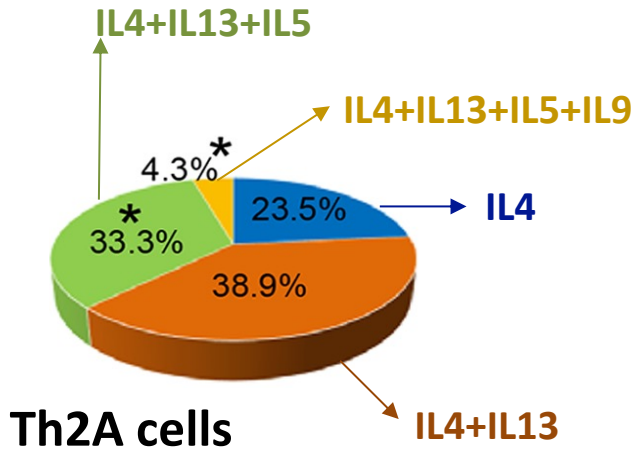
Antigen-specific Th2 cells



Pathogenic antigen-specific Th2A cells

CD45RB^{low} CD27- CRTH2+CD49d+ CD161+





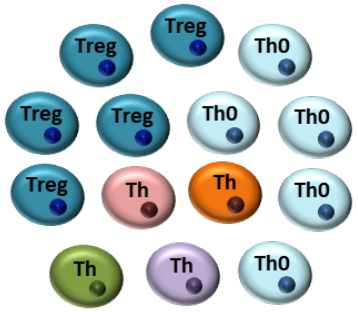
Polyfunctionality of Th2A cells with simultaneous expression of multiple Th2 effector cytokines

(Adapted from Wambre et al., 2017, Sci Transl Med, 9, eaam9171)

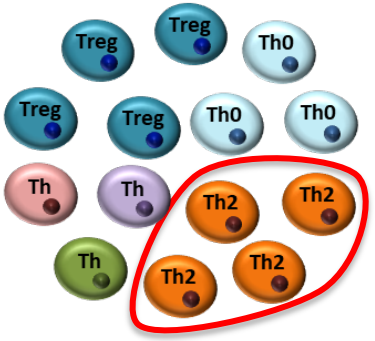
Detection of allergen-specific T cells

APPROACHES FOR THE STUDY OF ALLERGEN-SPECIFIC T CELLS

TOLERANCE



ALLERGY



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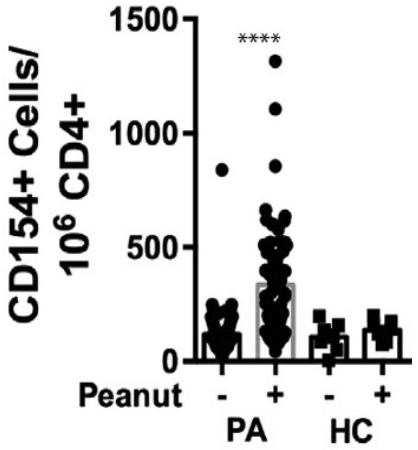
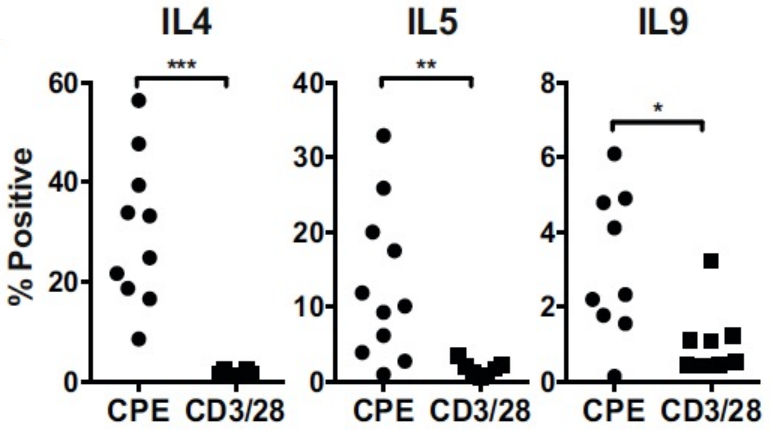
***In vitro* expansion**	Treg Th0 Th Th2 Th2	*Bystander activation* *Modifies % and phenotype*
Intracellular cytokines	Th Th2	*Non-cytokine-producing* *Naive T cells and Treg*
pMHC-II multimers	Treg Th0 Th Th2	*T cells subsets specific against unknown epitopes*
Activation markers	Treg Th0 Th Th2 Th2	

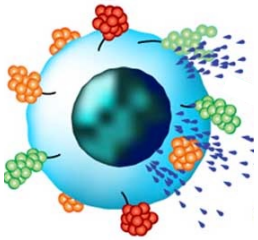
Identification of allergen-specific Th2 cells by ACTIVATION MARKERS



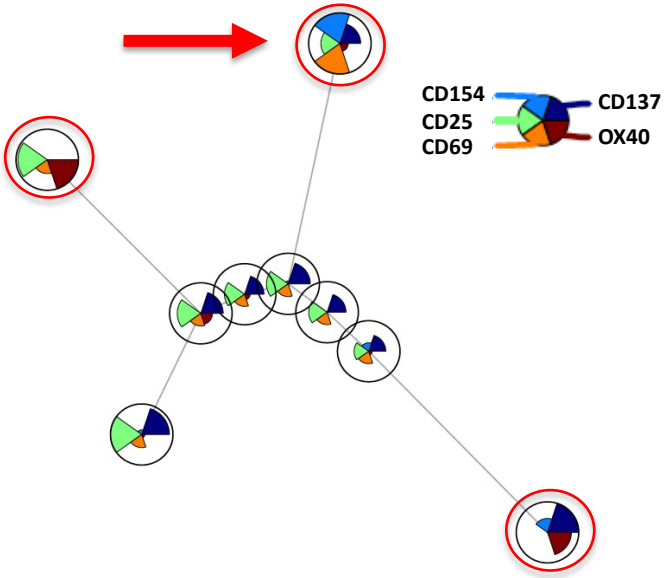
(Chiang et al., 2018, JACI, 141:2107-2120)



CD154 identified highly differentiated allergen-specific Th2 cells



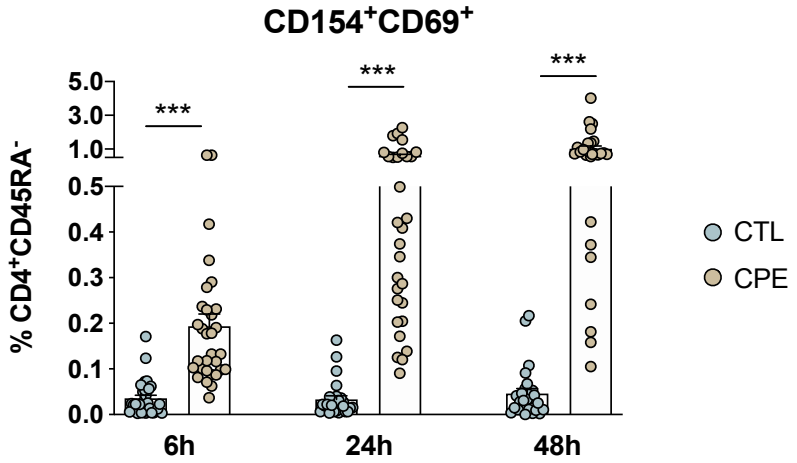
Induced <u>ACTIVATION MARKERS</u> for the identification of allergen- specific T cells:



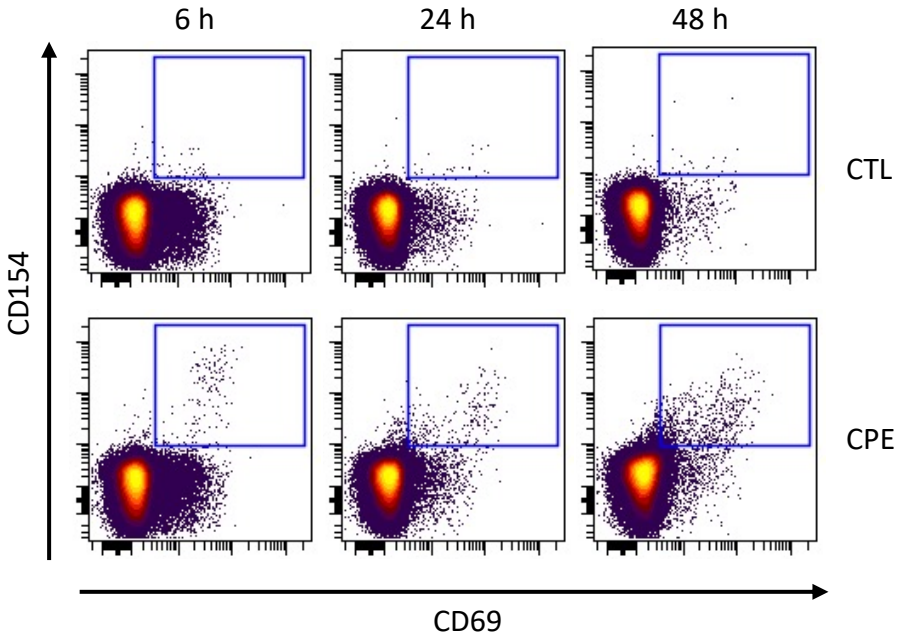
- ***Kinetics of expression of activation markers***
- ***Combined expression of activation***

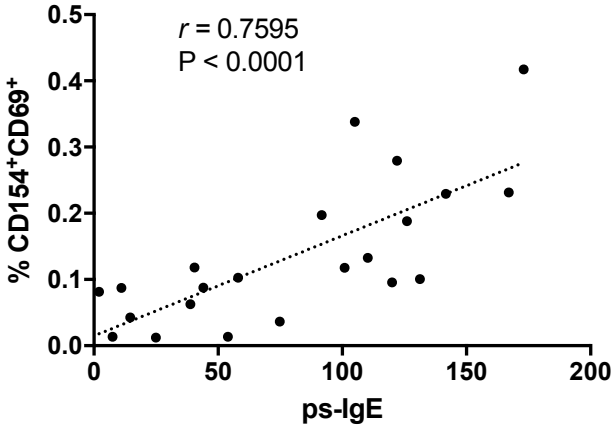
(Lozano-Ojalvo et al., 2021. Submitted)

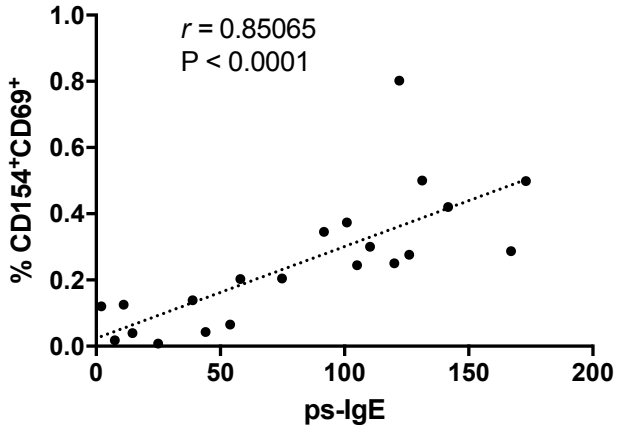
Identification of allergen-specific Th2 cells by ACTIVATION MARKERS

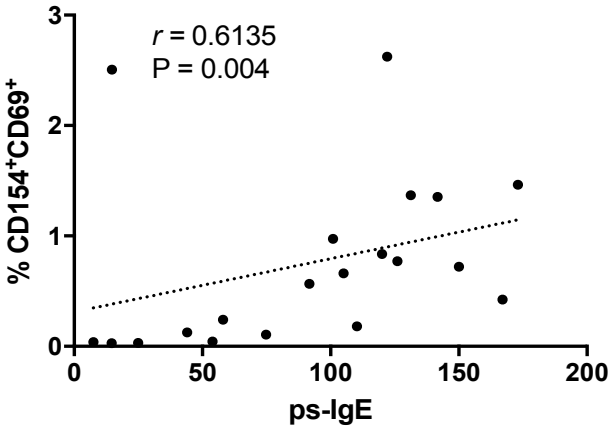


CD154/CD69 allow the early identification of allergen-specific T cells





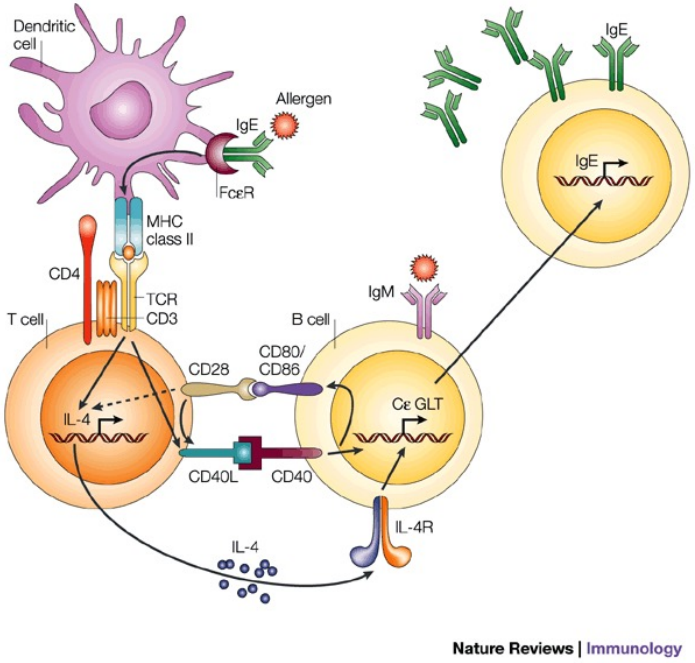




(Lozano-Ojalvo et al., 2021. Submitted)

In vitro models to assess B cell isotype switching (KE5)

Lack of *in vitro* methods for investigating B cell class-switching induced by food allergens



Challenge: To identify methods

(Geha et al., 2003, Nat. Rev. Immunol., 3, 721-732)

CONCLUSIONS

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Review

Applying the adverse outcome pathway (AOP) for food sensitization to support *in vitro* testing strategies

Daniel Lozano-Ojalvo[a,*], Sara Benedé[b], Celia M. Antunes[c], Simona L. Bavaro[d], Grégory Bouchaud[e], Ana Costa[c], Sandra Denery-Papini[e], Araceli Díaz-Perales[f], María Garrido-Arandia[f], Marija Gavrovic-Jankulovic[g], Simone Hayen[h], Mónica Martínez-Blanco[i], Elena Molina[i], Linda Monaci[d], Raymond H.H. Pieters[j], Clelia Villemin[e], Harry J. Wichers[k], Barbara Wróblewska[l], Linette E.M. Willemsen[m], Erwin L. Roggen[n], Jolanda H.M. van Bilsen[o]

- *In vitro* testing strategies for predicting allergenicity has been recently proposed
- B cell methods should be deeply investigated
- **Validate the *in vitro* methods** proposed and **harmonize** a strategy for predicting sensitization

PERSPECTIVES

EU Risk Assessment Agenda (EU RAA) database

- **Development of a harmonized strategy for the *in vitro* assessment of food allergenicity** (Spain)
- **Harmonized *in vitro* methods to evaluate dietary protein quality for human nutrition** (France)

EFSA Strategy topics:

- *SO1.2 Make documentation on information gathering and the evaluation process available*
- *S03.2 Growing the EU and international RA community*
- *SO4.2 Develop and implement harmonized methodologies and guidance documents for risk assessment across the EU and internationally*

- Determinations of allergen thresholds, measurements of allergens in food (Germany)
- Allergenicity of processed and raw food allergens and related analytical detectability (Belgium)
- Nanoplastic contamination in food: new factor affecting food allergy (Spain)

In vitro methods for predicting allergenicity

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Working Group 2: **In vitro *methods for predicting sensitization***