

Endpoint „Skin Sensitization“

**Sensitization testing
in the frame of REACH:
Any reliable *in vitro* alternatives in sight?**

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Outline

- I. Current knowledge on chemical sensitization (in skin)**
- II. Current developments in the field of *in vitro* testing**
- III. Prospects: When will reliable *in vitro* tests be available?**

Current knowledge on skin sensitization

in brief

Allergic Contact Dermatitis (ACD)

Issues and Implications

- **3000-5000 chemical contact allergens („elicitors“)**
- **Incidence_{dermat. clinic}: 2-7/1000/year**
- **Lifetime prevalence: ~15% (Survey 2000)**
- **Occupational dermatoses: ~25%**
- **Psychological strain/persistence/occupational disability**
- **Multifactorial disease**

Major Challenges

EU Cosmetics Directive 7th Amendment

- Marketing ban since 2003 for testing finished products in animals
- Phasing out of testing in animals and stepwise marketing ban in 2009 and 2013



REACH Legislation in the EU

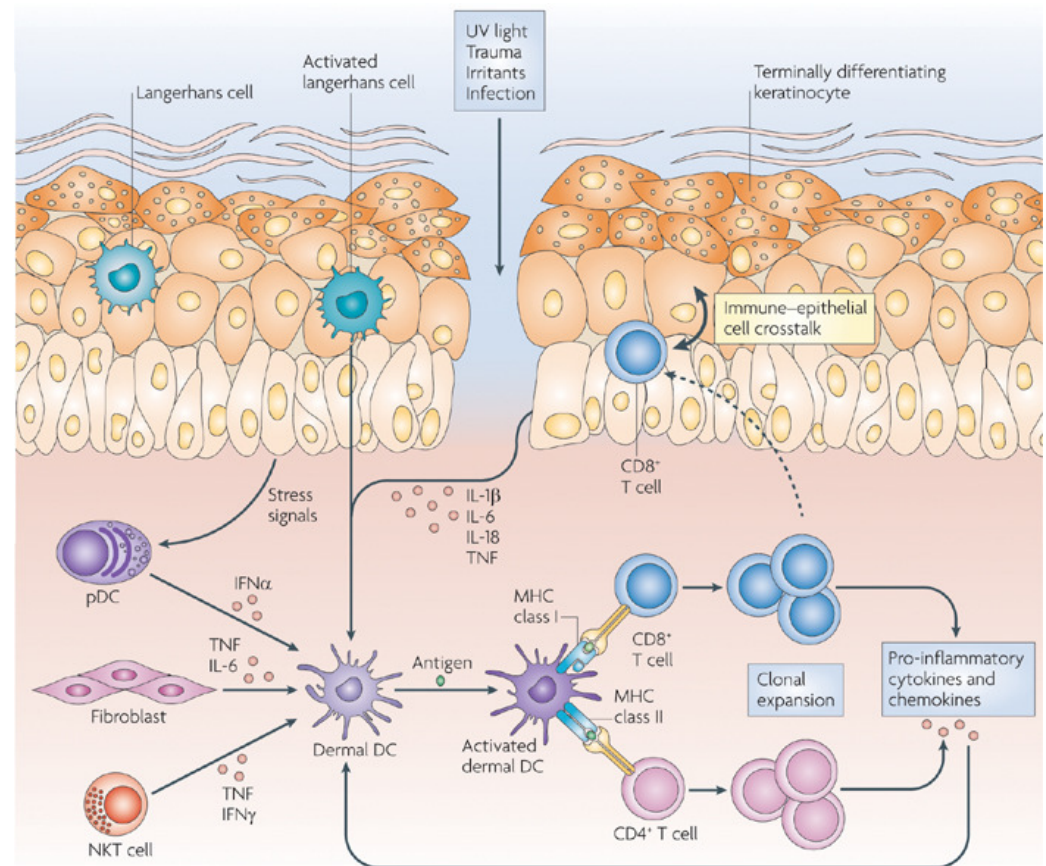
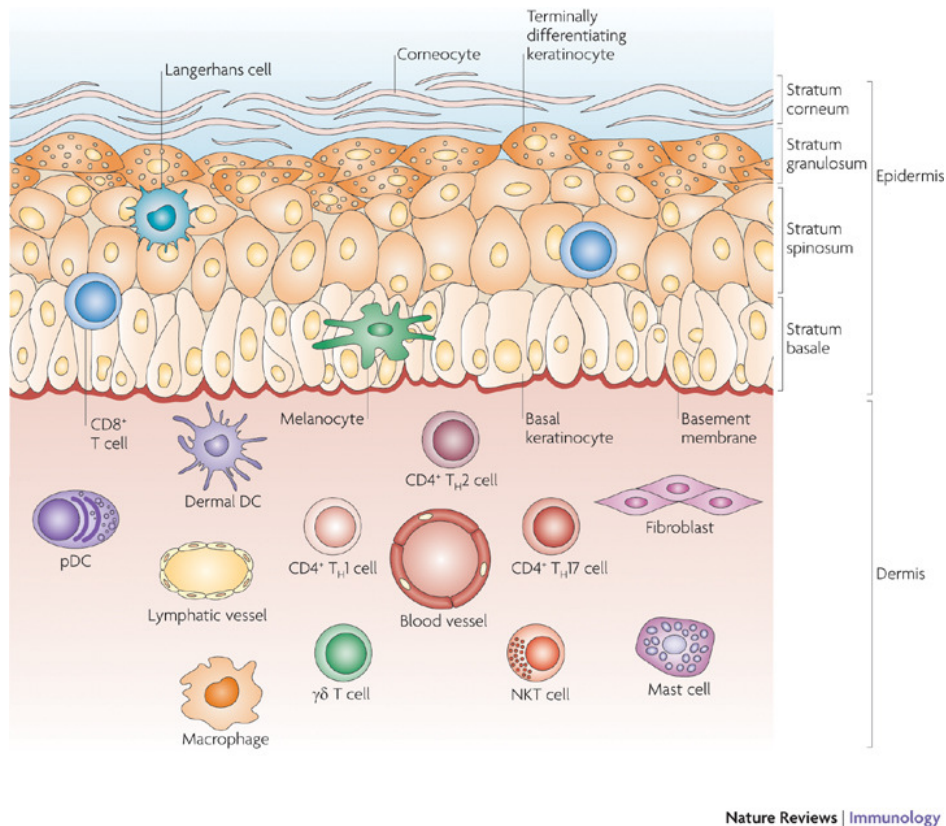
- 30,000 chemicals beyond volume of >1 ton/year require toxicological evaluation
- 70% of testing being conducted between 2011-2017

Toxicological Safety Evaluation

- Animal experiments skin for sensitization/irritation (EU-25, 2005): ca. 60,000 animals
[skin sensitization: 22,184 guinea pigs and 21,350 mice]
- Current test systems based on alterations of phenotype and cytokine/chemokine release of dendritic cells

Ian Kimber et al. (2001) Toxicol Sci 59: 198-208

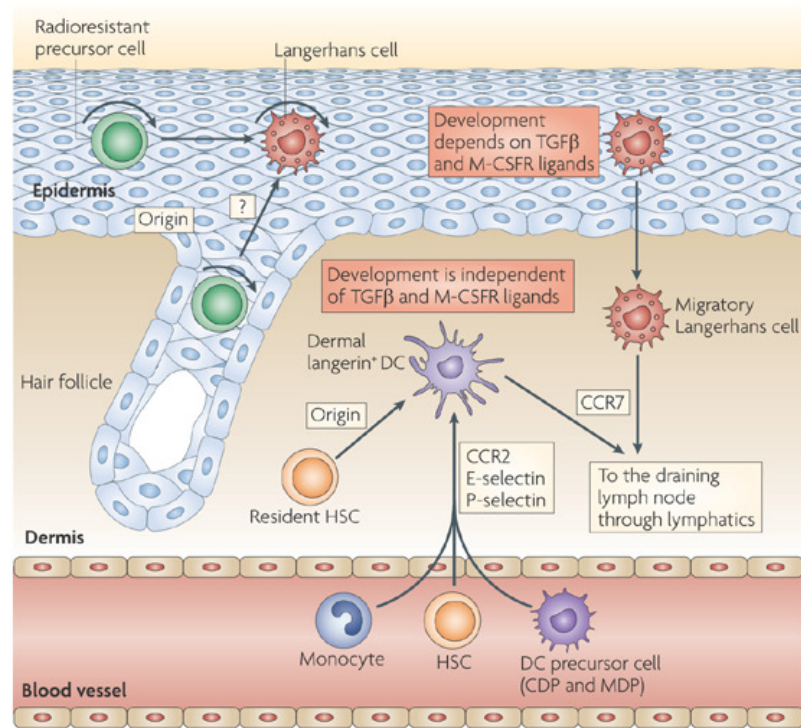
Skin Anatomy and Cellular Effectors



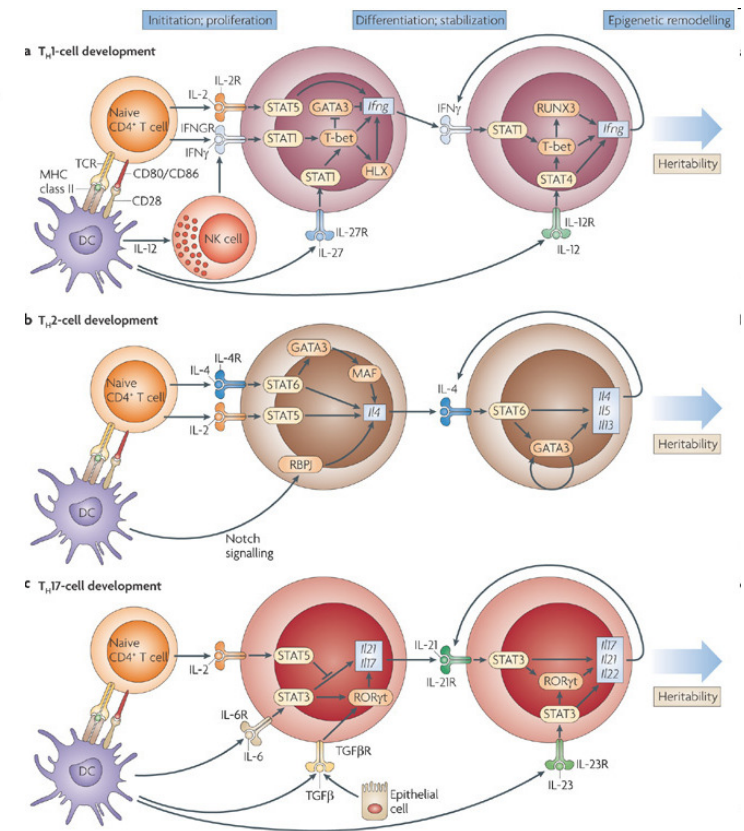
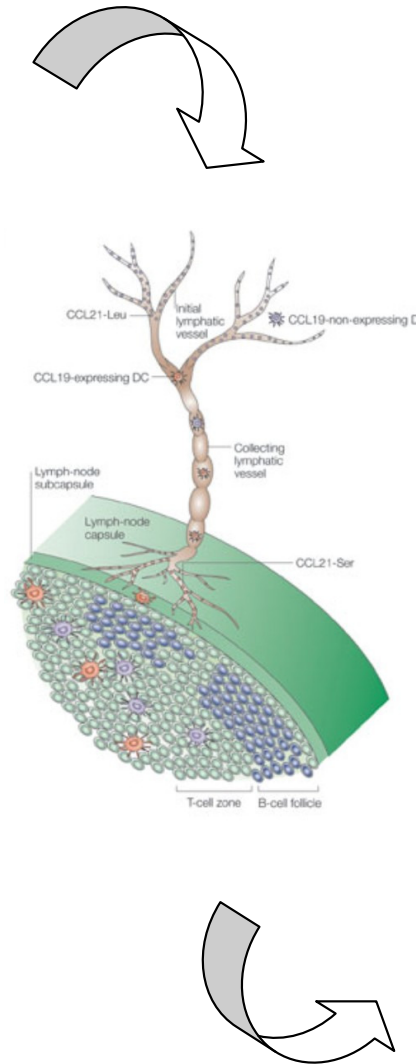
Frank O. Nestle et al. (2009) NRI 9: 679-691

Gwendalyn J. Randolph et al. (2005) NRI 5: 617-628

DC Homeostasis, Migration & T-Cell Interaction



Nature Reviews | Immunology

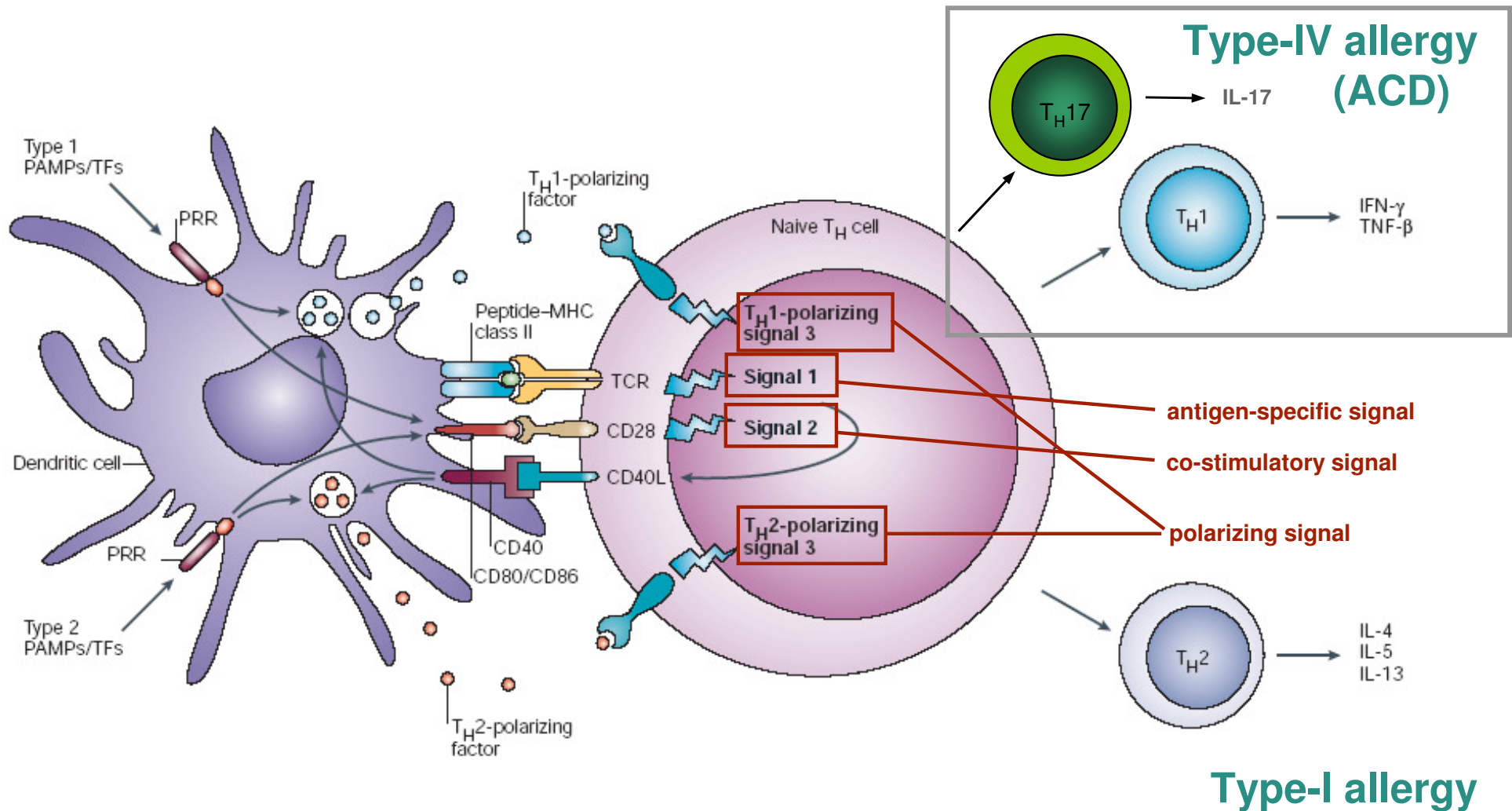


Nature Reviews | Immunology

Miriam Merad et al. (2008) NRI 8: 935-947

Gwendalyn J. Randolph et al. (2005) NRI 5: 617-628

T-Cell Stimulation and Polarization



Martien L. Kapsenberg (2003) NRI 3: 984-993 (modified)

Biological Endpoints?

- Immune cell migration
- Allergen presentation in lymph node
- **Proinflammatory cytokine/chemokine release**
- T cell differentiation
- Tissue damage (cell death)

Irritation vs Sensitization: Predictive biomarkers?

Skin irritating compound:

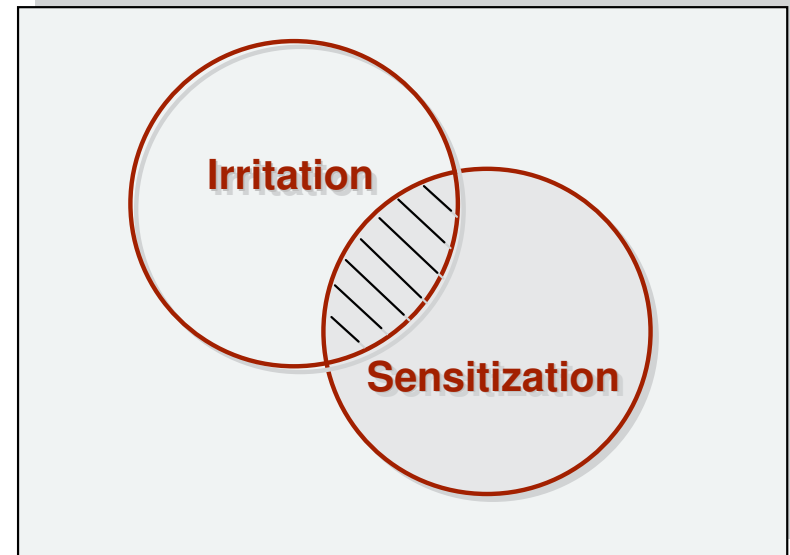
IL-1 α , IL-8, IL-18, IL-6, IL-10, TNF α , LIF

Skin sensitizing compound:

Sensitization phase: CD86, MHC-II, CD54, CD58, IL-1 β , IL-18, IL-12, IL-6, TNF β , ATP, LTB₄, ROS, histamine, PGE₂, histamine, CCL2, IgM (liver)

Elicitation phase: IL-1 β , IL-18, IL-6, IL-10, TNF β , ATP, IFN- γ , IL-17

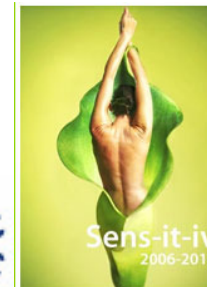
Resolution phase: IL-10, TGF- β



Current developments in *in vitro* testing ?

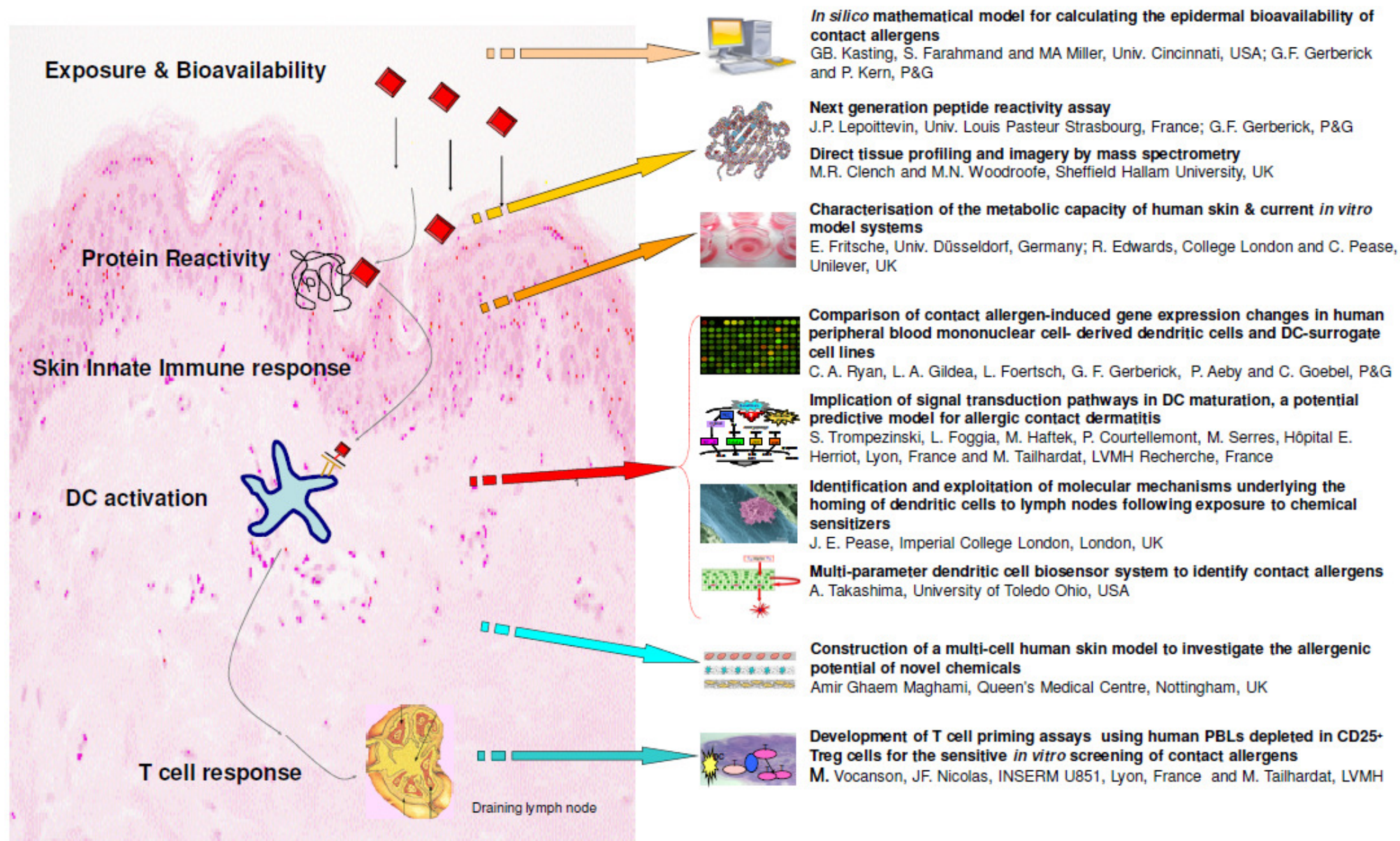


COLIPA
Sens-it-iv
BfR



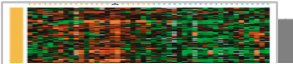
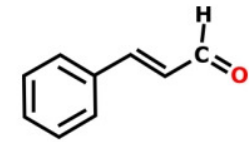
Risiken erkennen – Gesundheit schützen

COLIPA Skin Tolerance Task Force: Portfolio of research projects



Expression profiling of MoDCs and MUTZ-3 after 24 h cinnamaldehyde

↑ 1741
 ↓ 23
 731
 855
 Ø 72
 Ø 8

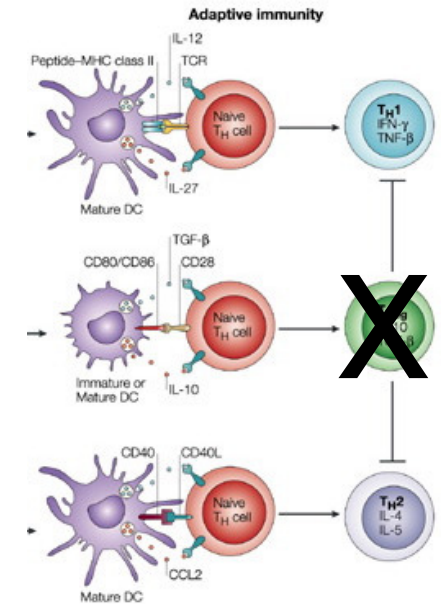


Biological processes classification of genes significantly up-regulated by CIN in both PBMDs and MUTZ-3 cells.

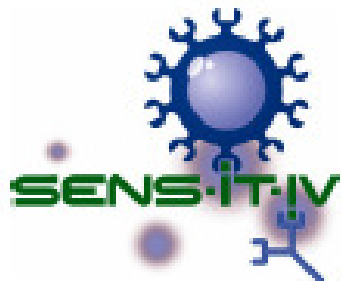
Accession no.	Gene name	Gene symbol	FC induced by CIN	
			PBMDCs	MUTZ-3 cells
Apoptosis-related (3)				
NM_006410	HIV-1 Tat interactive protein 2, 30 kDa	HTATIP2	1.66	2.05
NM_003311	Pleckstrin homology-like domain, family A, member 2	PHLDA2	2.14	2.90
NM_014452	Tumor necrosis factor superfamily, member 21	TNFRSF21	2.18	2.44
Cell adhesion-related (3)				
NM_003812	ADAM metalloproteinase domain 23	ADAM23	6.06	2.87
NM_000094	Collagen, type VII, alpha 1	COL7A1	2.03	2.82
NM_177444	PTPRF interacting protein, binding protein 1 (liprin beta 1)	PPFIBP1	1.80	2.54
Cell differentiation-related (1)				
NM_001430	Endothelial PAS domain protein 1	EPAS1	3.09	3.05
Endocytosis-related (1)				
NM_013437	Low density lipoprotein-related 12	LRP12	1.95	2.24
G-protein-related (1)				
NM_005294	G-protein-coupled receptor 21	GPR21	2.27	2.72
Immune response/inflammatory response-related (8)				
NM_006889	CD86 molecule	CD86	1.70	2.47
NM_004001	Fc fragment of IgG, low affinity IIb, receptor (CD32)	FCGR2B	2.04	2.16
NM_201563	Fc fragment of IgG, low affinity IIc, receptor for (CD32)	FCGR2C	2.06	3.04
NM_002032	Ferritin, heavy polypeptide 1	FTH1	1.53	2.90
NM_139010	Hemochromatosis	HFE	2.68	2.60
NM_000576	Interleukin 1, beta	IL1B	1.97	2.06
NM_000584	Interleukin 8	IL8	1.67	2.10
NM_138554	Toll-like receptor 4	TLR4	2.12	2.49

Francois Python et al. (2009) TAAP 239: 273-283

T-Cell Priming Assay co-incubating DCs & T_{reg}-depleted lymphocytes



Development of T cell priming assays
using human PBLs depleted in CD25⁺ Treg cells
for the sensitive *in vitro* screening of contact allergens



Novel testing strategies for *in vitro* assessment of allergens

EU FP6, "Sens-it-iv", 2005-2010

WP1:
**Database of
reference compounds**

WP2:
**EC-DC Interactions -
Cellular responses**

NCTC IL-18 assay
DC migration assay
3D skin models

WP3:
**T cell-based assays
DC-T cell interaction**
In vitro T cell priming assay
T cell amplification assay

WP4:
Genomic analysis
Identification of
new biomarkers

WP5:
Proteomic analysis
Identification of
new biomarkers

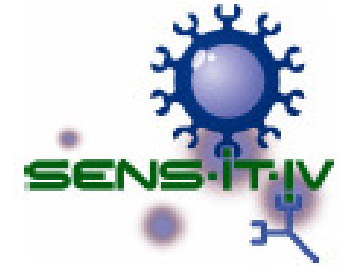
WP6:
Metabonomic analysis
Metabolic activation of
pro- and pre-haptens

WP7:
Data management

WP8:
***In vitro* assay development**
Technology transfer
Round robin

WP9:
**Dissemination of
knowledge**
Public relations

WP2: EC-DC Interactions - Cellular responses



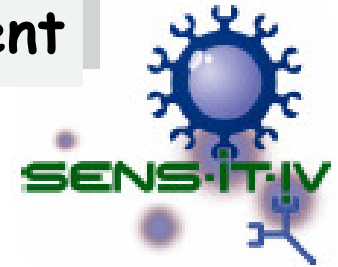
WP2B: Finding the most in vivo-like epithelial cell line and EC markers: IL-8, IL-6, CD47, CD54 and CXCL5

WP2D: Finding the in vitro conditions supporting the most in vivo-like EC-DC interactions: MUTZ-3 in coculture with Calu-3 in an airlifted two-layer system transwell system, Episkin epidermis and skin epidermal equivalents

Deliverables

- a)** Characterization of the lung epithelial cells and available epithelial cell lines and identification of the most in vivo-like cell line, using techniques for protein analysis.
- b)** Establishment of protocols for optimal culture conditions for epithelial cell lines.
- c)** Determining the effect of allergens on lung epithelial cells and cell lines in terms of function, protein expression and metabolism. Identification of markers involved in the initiation of allergic responses.
- d)** Genome-wide comparison of tissue (skin, lung, tonsils) dendritic cells, primary cell-derived dendritic cells and cell lines. Identification of the most in vivo-like dendritic cells.
- e)** Determining the effect of allergens on dendritic cells in terms of function, gene expression, protein expression and metabolism. Identification of markers involved in the initiation of allergic responses.
- f)** Establishing a 3-dimensional model of epithelial cell-dendritic cell interaction using cell lines.
- g)** Incorporation of T cells into the 3-dimensional model. Identification of changes in gene and protein expression in interacting epithelial cells and dendritic cells after allergen stimulation, induced by selected T cell populations.

WP3/8: DC-TC Interaction / *In vitro* Assay Development



WP3: Establishing and implementing tools for addressing DC – T-cell interactions: T cell based assay capable of identifying contact allergens

Deliverables

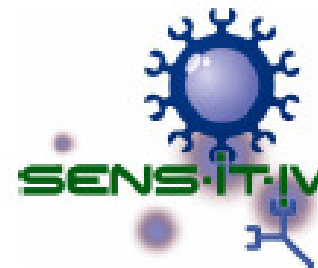
- a) Phenotypic, genomic and proteomic signatures of circulating and tissue infiltrating effector and memory T cells specific for the selected compounds (chemicals and proteins) and identification of immunodominant T cell epitopes in the same compounds.
- b) Definition of compound-interacting proteins as mediators for innate and adaptive immune responses of T cells, DCs and ECs.
- c) Definition of compound-driven changes on tissue cell types, DC subsets, T cell homing and polarization.
- d) Development of predictive assays to assess allergenicity of novel compounds.

WP8: *In vitro* assay development: MUTZ-3, U937 and THP-1 and biomarkers CD86 and IL-8

Rationale

“The aim of WP8 is to develop *in vitro* assays by improving existing assays for sensitization using innovations in the area of cell culturing and novel marker.”

Example:



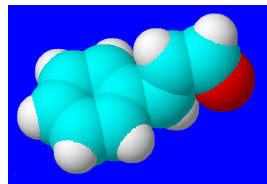
In vitro T cell priming assay for prediction of antigenicity



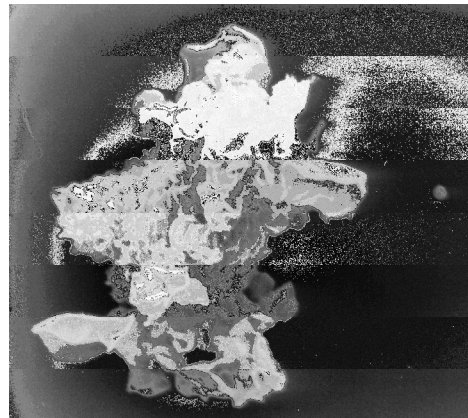
Dietz, L. et al., *manuscript in preparation*

„Contact Allergen Activated T-Cell (CAATC)-Assay“ using dendritic cells from skin: characterization of the sensitizing potency of chemicals via dendritic cell-induced expression of lineage specific T cell transcription factors

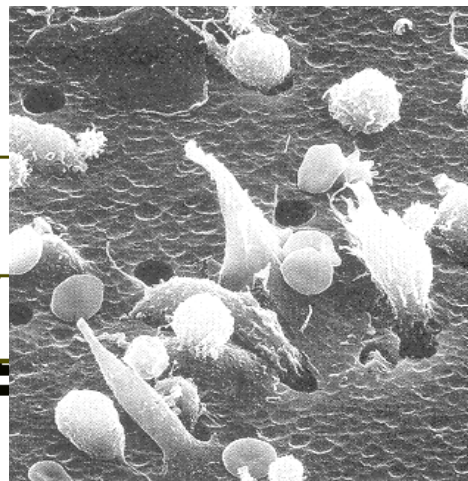
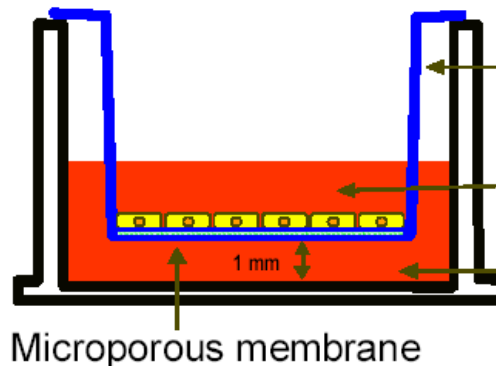
Contact allergen



Stimulation

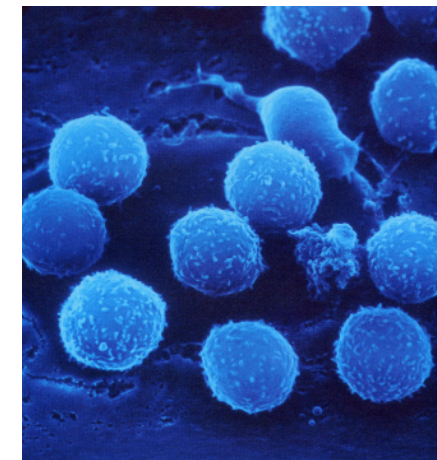


Dendritic Cell



Co-culture

T-helper cell (T_H)



CAATC Analysis

Transcription
factors

Cytokines

Endpoints:
Ratio

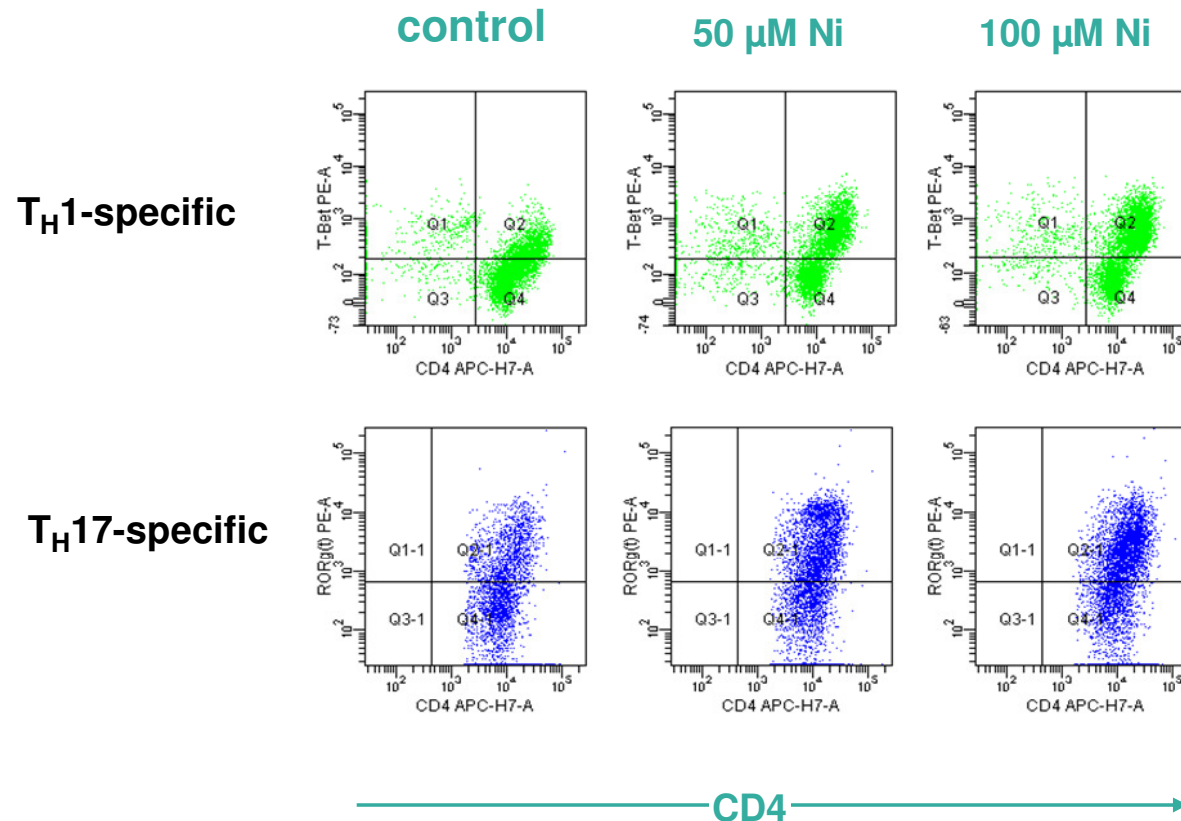
% pos. cells
ng/ml

„Contact Allergen Activated T-Cell (CAATC)-Assay“

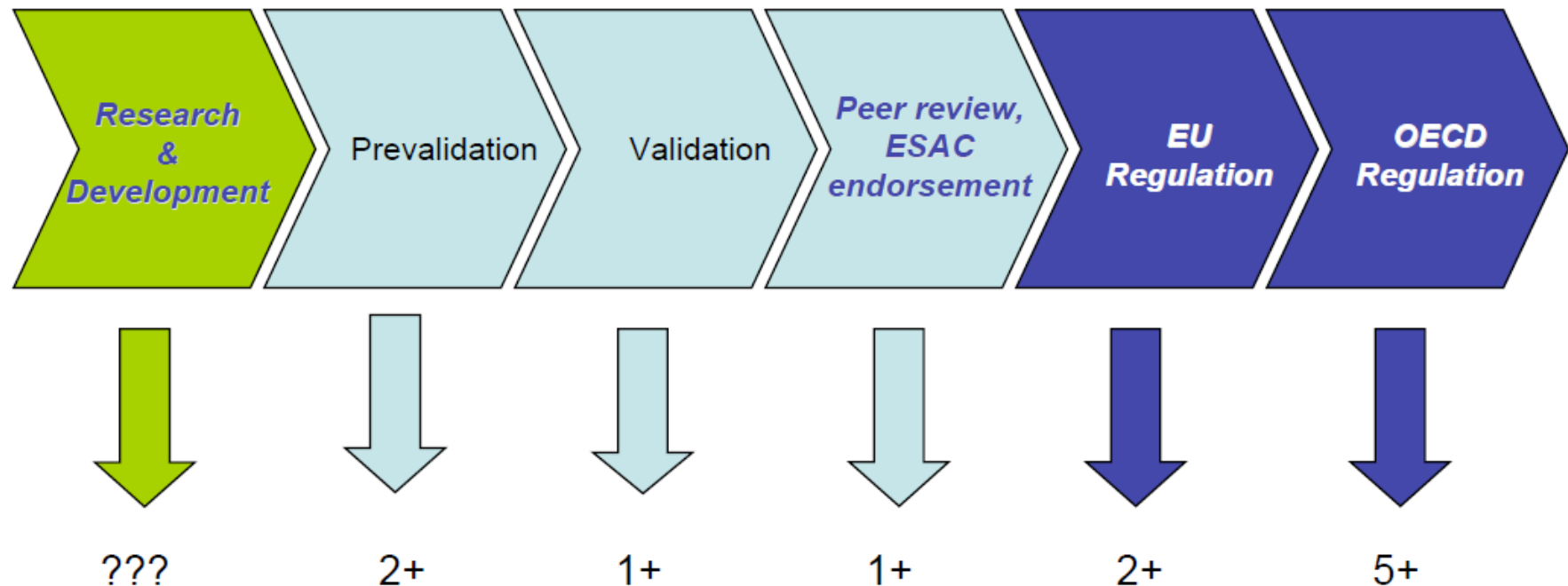
New assay to detect T-cell polarization

- Immune cells from skin desirable, skin exposed *in vivo*
- Dendritic cells directly exposed to chemical allergen
- Coculture of migrated dendritic cells with naïve T cells
- Identification of **Biomarkers** in polarized T_H cells
- Detection of **Proliferation** in T cells, T_H1, T_H17

BfR: DC-induced T cell transcription factors *in vitro*



Prospects: When will reliable *in vitro* tests be available?



Irene Manou et al. (2005) *Altern Lab Anim* 33, S1: 21-26.

Example: Murine LLNA on its way toward a test guideline

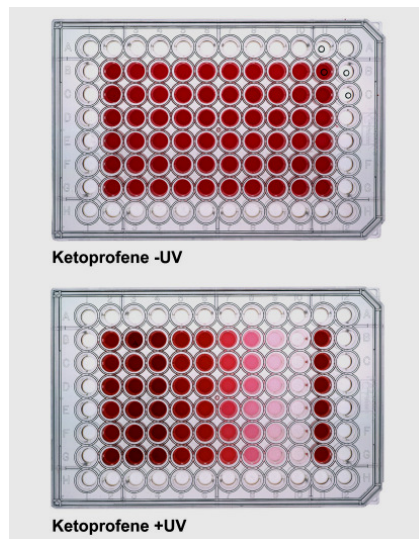
18 years to regulatory acceptance



1984	LLNA conceived
1986	First paper
1987-1990	Interlaboratory development
1992	Publication of standard protocol
1989-1997	Interlaboratory validation
1990-1996	Comparison with guinea pig database
1992-1996	Comparison with human data
1997-1998	Regulatory review
2002	Adopted as OECD TG 429

Example: Accelerated acceptance of 3T3 NRU Phototox

6 years to regulatory acceptance



1994	3T3 NRU phototoxicity test published
1992-1994	Prevalidation
1995-1997	Validation
2000	EU Annex V 67/548 EEC
2004	Adopted as OECD TG 432
2008	Council Regulation on REACH Test Methods

What is currently in the pipeline?

OECD:

- Two modified versions of traditional LLNA (non-radioactive protocols)
- TG 429 update
- Two updates for “*In vitro* skin Corrosion“, TG 430/431
- New TG *in vitro* test for skin irritation

→ **No *in vitro* sensitization assay (in 2009)**

ECVAM validation:

- „Direct binding peptide reactivity assay“
- hCLAT assay (human cell line activation test: CD54/86 @ THP-1)
- MUSST assay (myeloid U937 skin sensitization test: CD86 @ U937)

**Thank you
for your attention !**

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