New immunological tests to identify skin sensitizers

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Research activities

Pathophysiological research

Skin allergic diseases


Allergic contact dermatitis (ACD) **Atopic dermatitis (AD)**

ECZEMAS


MILD - Exanthema **SEVERE – Blistering disease**

DRUG ALLERGIES

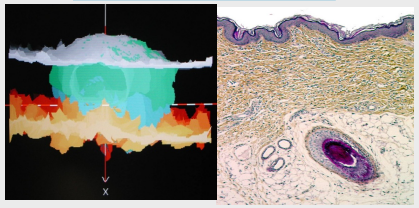
Translational research

New immunological assays

Diagnosis

Prediction of allergenicity

Intradermal vaccination

Part 1

Pathophysiology of Allergic Contact Dermatitis

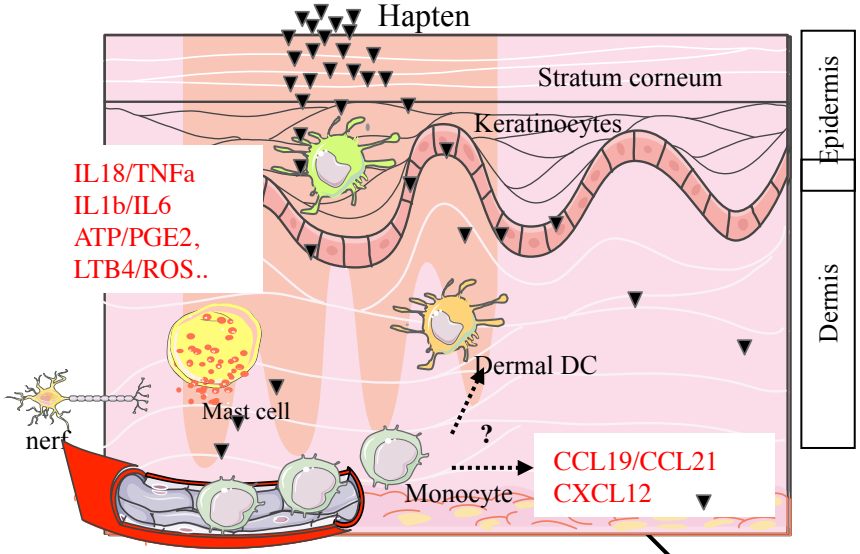
Murine models

(experimental or clinically relevant allergens)

Pathophysiology of ACD

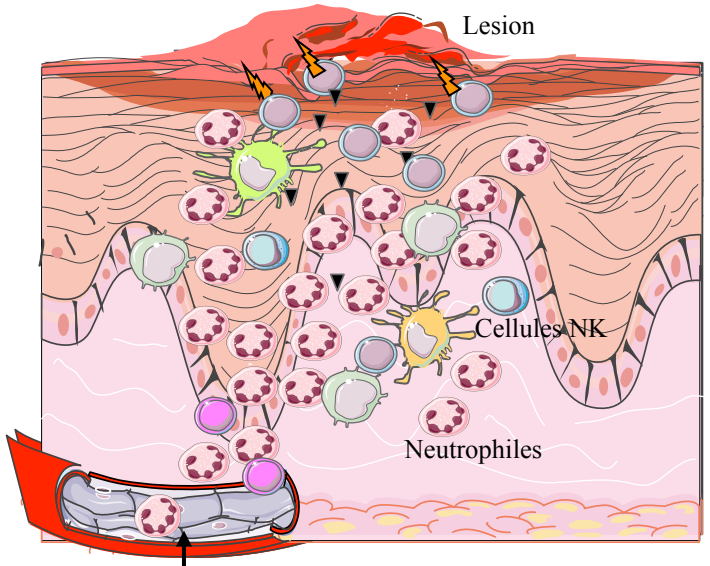
1- Sensitization phase

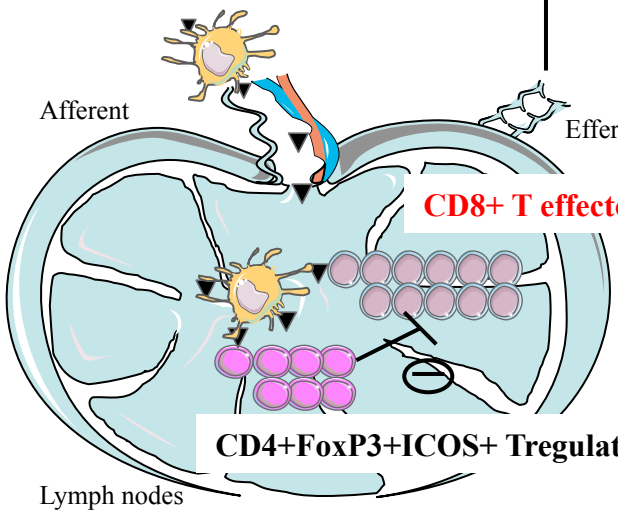
Innate response / T cell activation



2- Elicitation (challenge) phase

T effector response / Skin inflammation





Hélène BOUR et al., Eur J Immunol, 1995
Maya KRASTEVA et al., J Immunol, 1998
Jeanne KEHREN et al., J Exp Med, 1999
Hitoshi AKIBA et al., J Immunol, 2002
Pierre SAINT-MEZARD et al,. J Immunol, 2003
Marc VOCANSON et al., J Invest Dermatol, 2006
Marc VOCANSON et al., Allergy, 2009
Marc VOCANSON et al., JACI, 2010

Experimental model of ACD / Effector mechanisms

The Mouse Ear Swelling Test (MEST) to DNFB

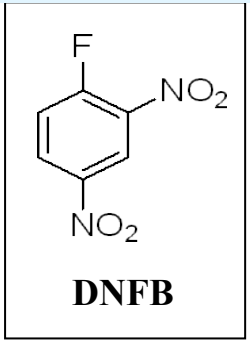
Sensitization
0.5% DNFB (Day 0)

5 days

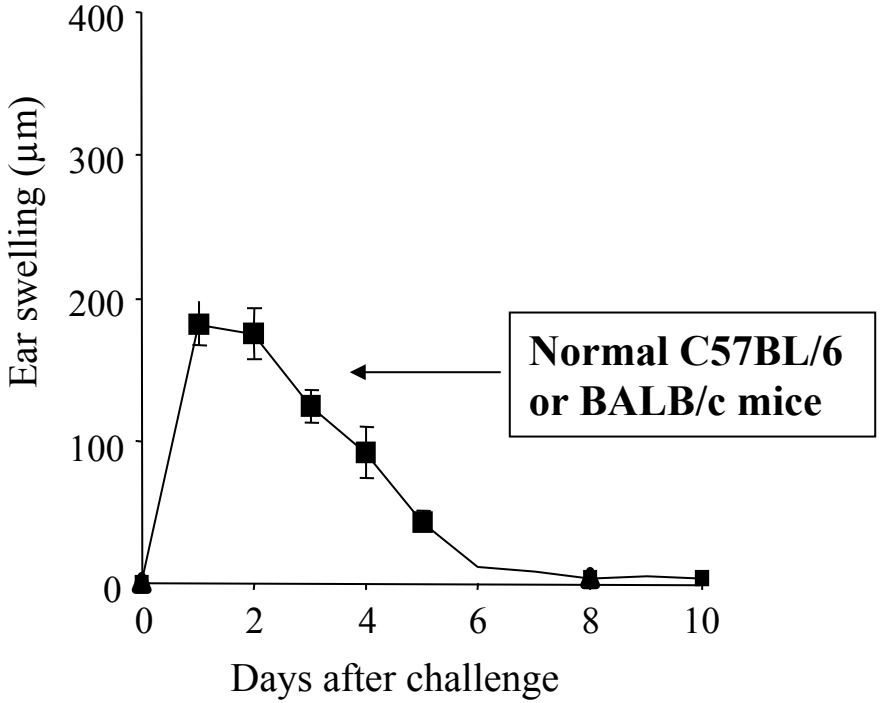
Challenge
0.2% DNFB (Day 5)

24-120 h

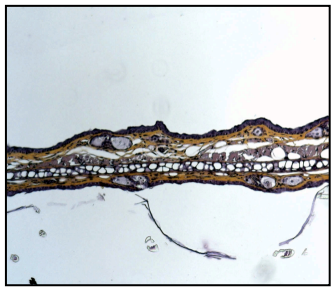
Ear swelling measurements



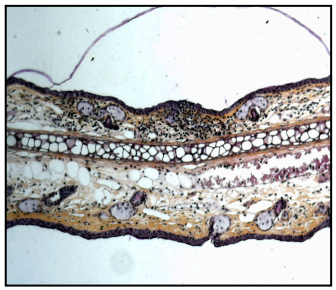
DNFB



Histology



Unsensitized



Sensitized

Experimental model of ACD / Effector mechanisms

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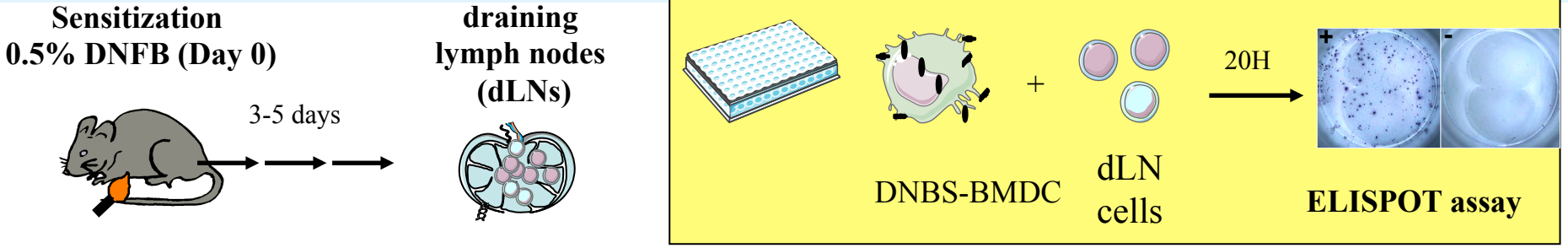
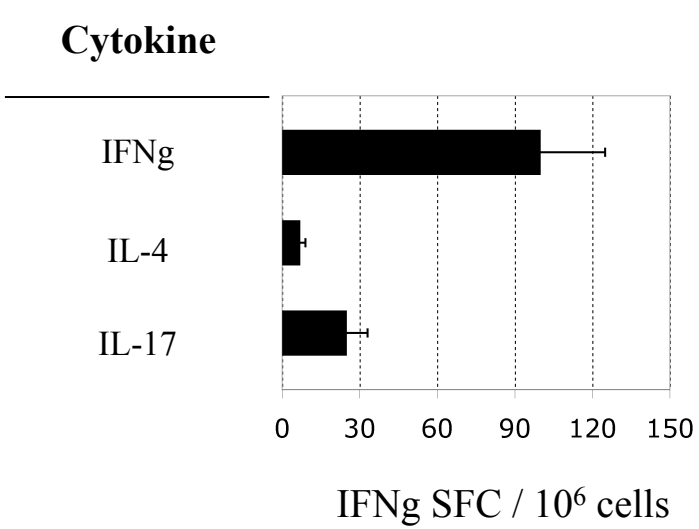
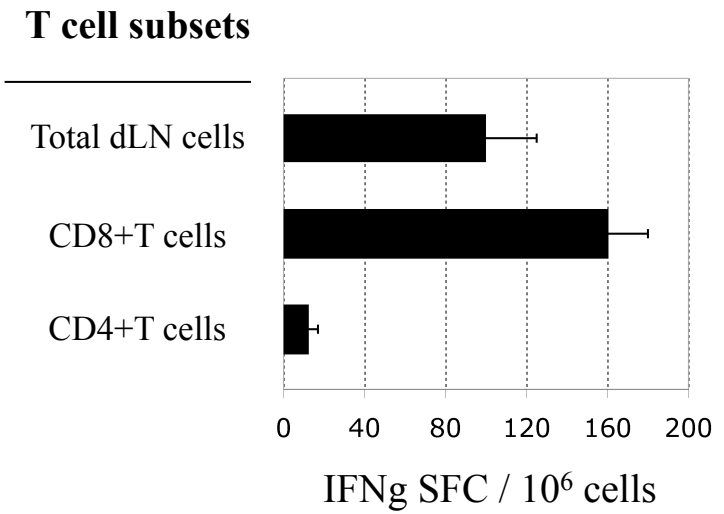


DNFB-specific T cell response

Major production of IFNg – mainly CD8+ T cells





Experimental model of ACD / Effector mechanisms

The Mouse Ear Swelling Test (MEST) to DNFB

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5 days

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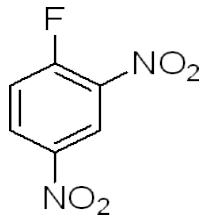
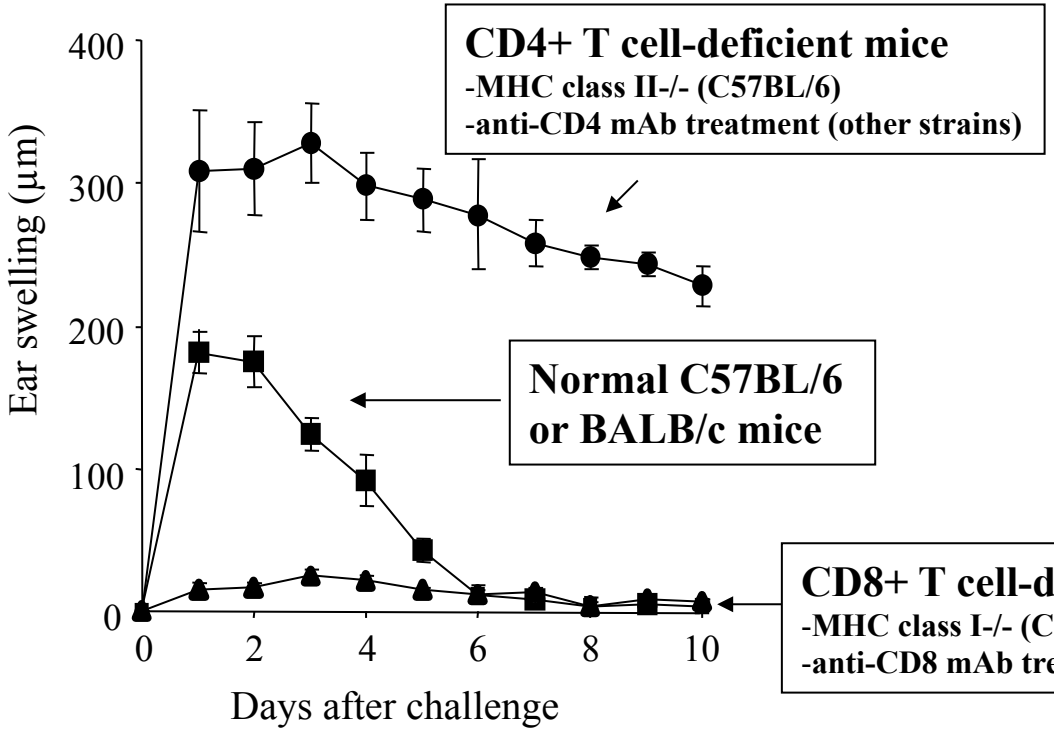
24-120 h

Ear swelling measurements



DNFB

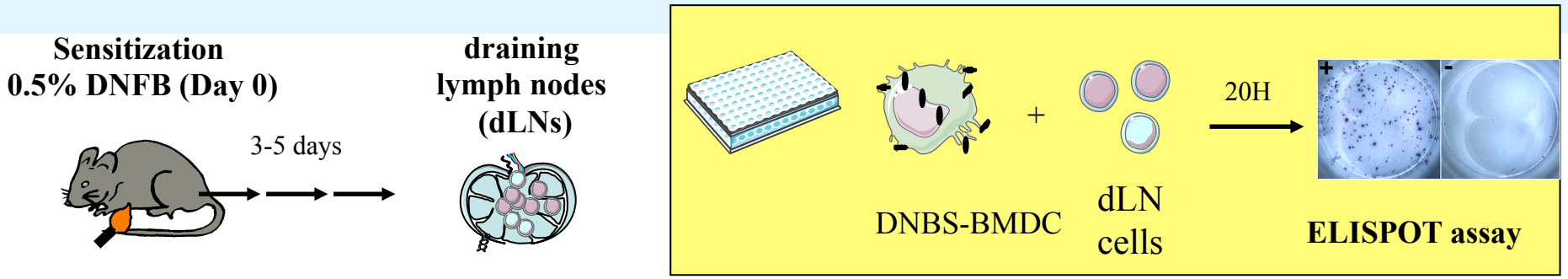


CD8+ T cells are effector cells /

CD4+T cells are regulatory

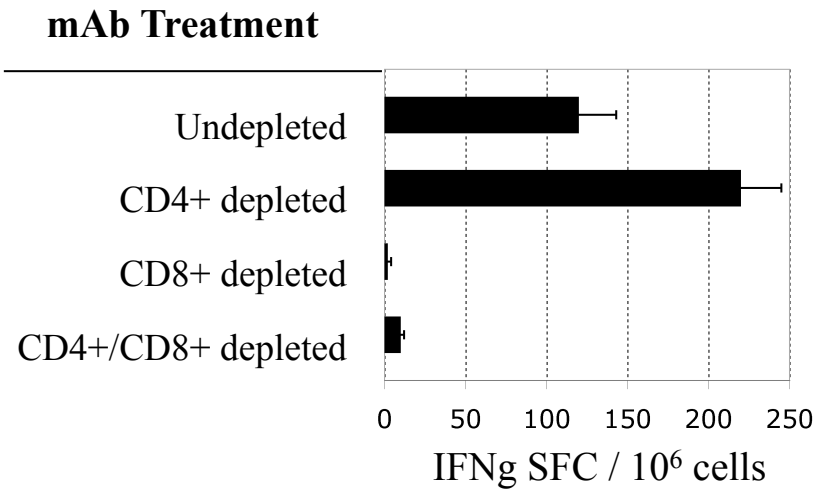
Experimental model of ACD / Effector mechanisms

The Mouse Ear Swelling Test (MEST) to DNFB



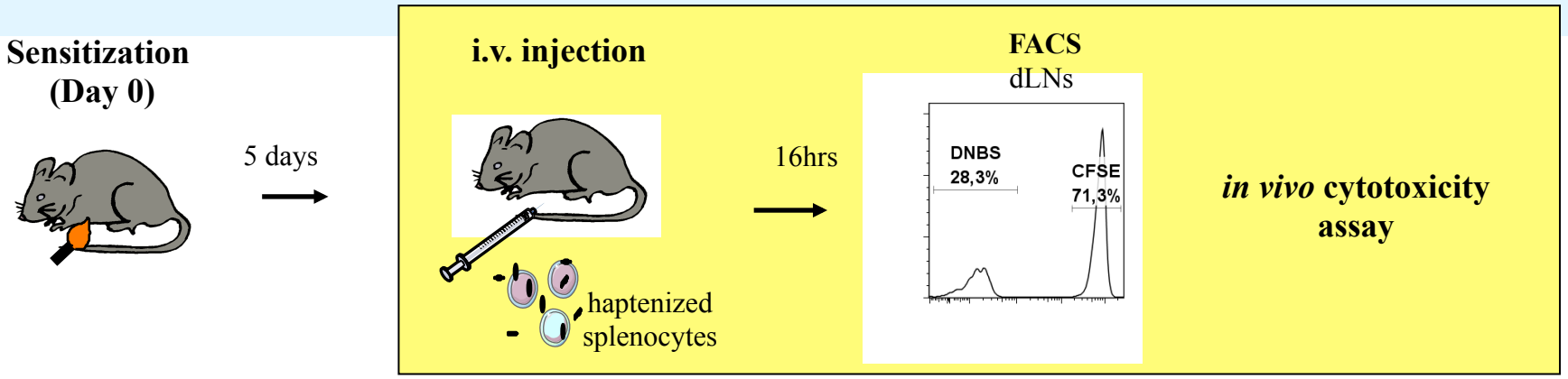
DNFB-specific T cell response

No production of IFNg in absence of CD8+ effector T cells

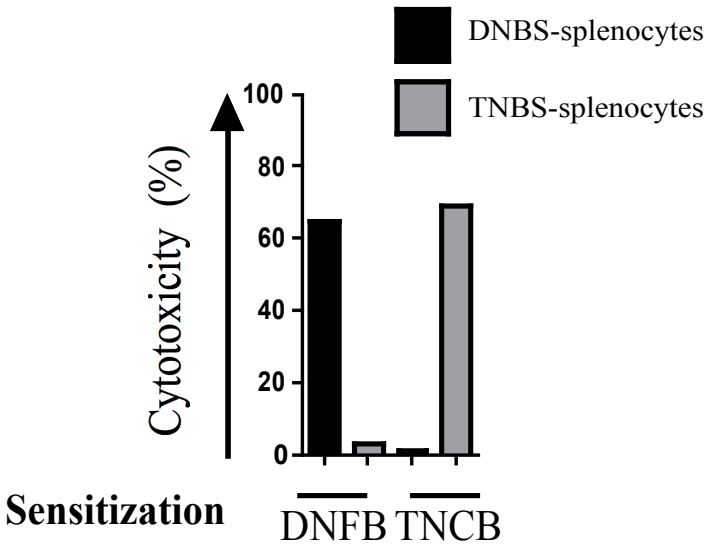


Experimental model of ACD / Effector mechanisms

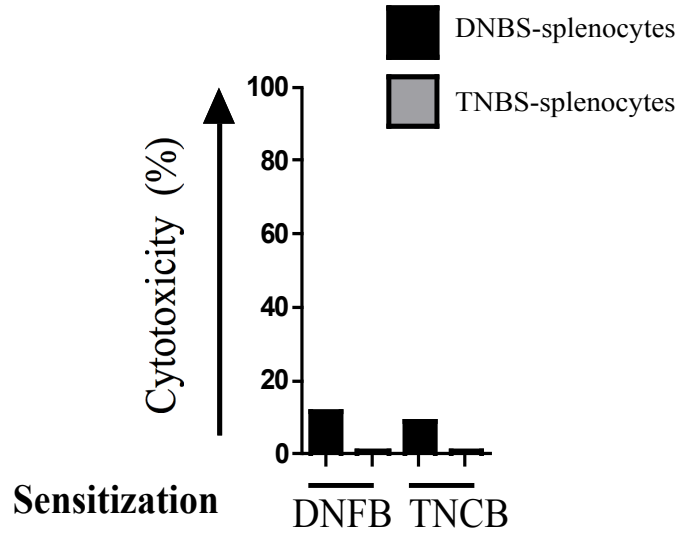
The Mouse Ear Swelling Test (MEST) to DNFB



Haptenized-targets are not killed in absence of CD8+ effector T cells



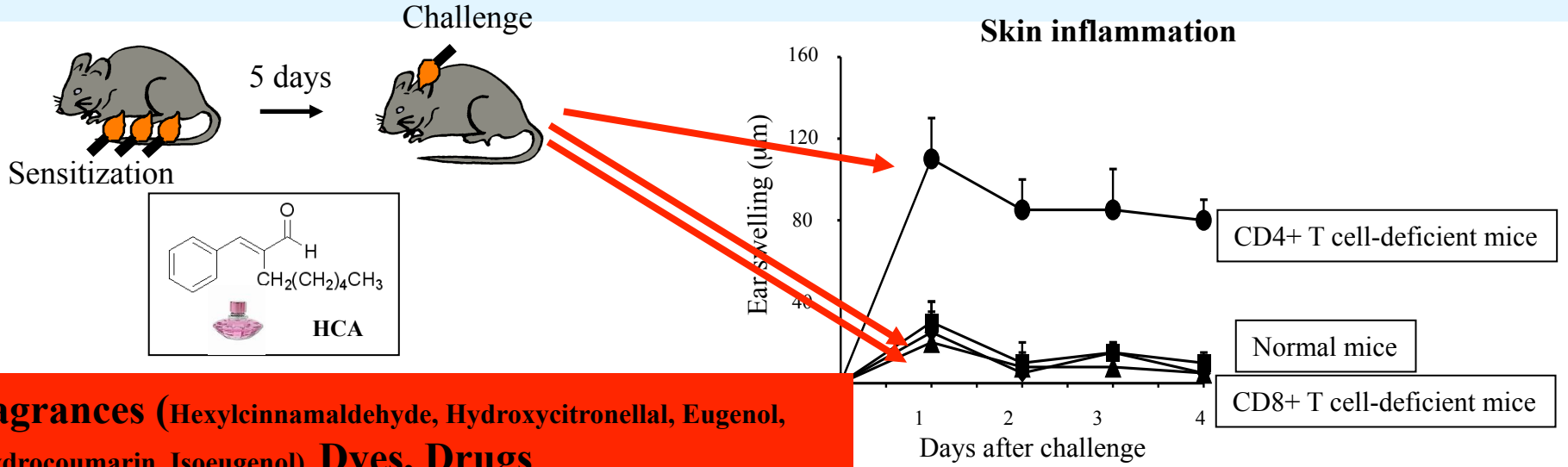
CD8+-depleted



Sensitizing potency of haptens

Chemical	Field	Sensitizing potency
Oxazolone	Chemistry	Extreme
2,4-Dinitrofluorobenzene	Chemistry	Extreme
2,4-Dinitrochlorobenzene	Chemistry	Extreme
Glutaraldehyde	Preservative, antiseptic	Strong
Formaldehyde	Cosmetic, Dye	Strong
Cinnamaldehyde	Perfum, Flavour	Moderate
Hexyl cinnamaldehyde	Cosmetic (perfum)	Moderate/weak
Eugenol	Cosmetic, antiseptic	Weak
Hydroxycitronellal	Cosmetic (perfum)	Weak
Linalool	Cosmetic (perfum)	Weak
Citral	Perfum, Flavour	Weak
Vanillin	Perfum, Flavour	Weak
Propyl paraben	Cosmetic	Weak
Amoxicillin, cyanamid, cetrimide	Drug	Weak

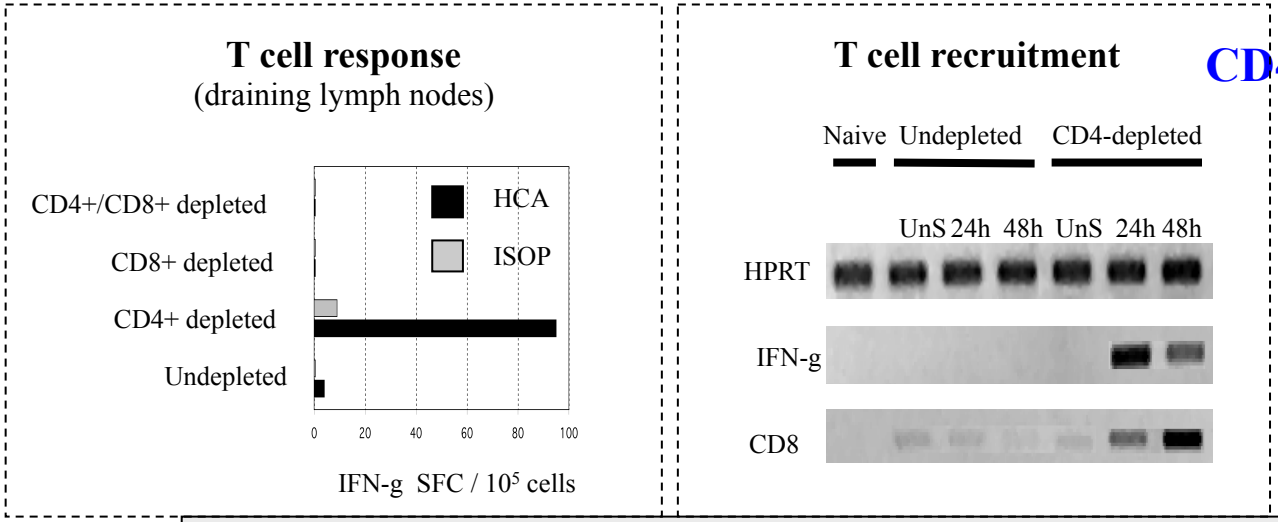
Mouse models of ACD and drug allergy to clinically relevant allergens



Fragrances (Hexylcinnamaldehyde, Hydroxycitronellal, Eugenol, Dihydrocoumarin, Isoeugenol), Dyes, Drugs

CD8+ T cells are effector cells

CD4+ T cells are tolerogenic



Vocanson M et al. J Invest Dermatol, 2006. / Laas C. et al. Exp Dermatol, 2008. / Rozières et al. Allergy 2010

Part 2

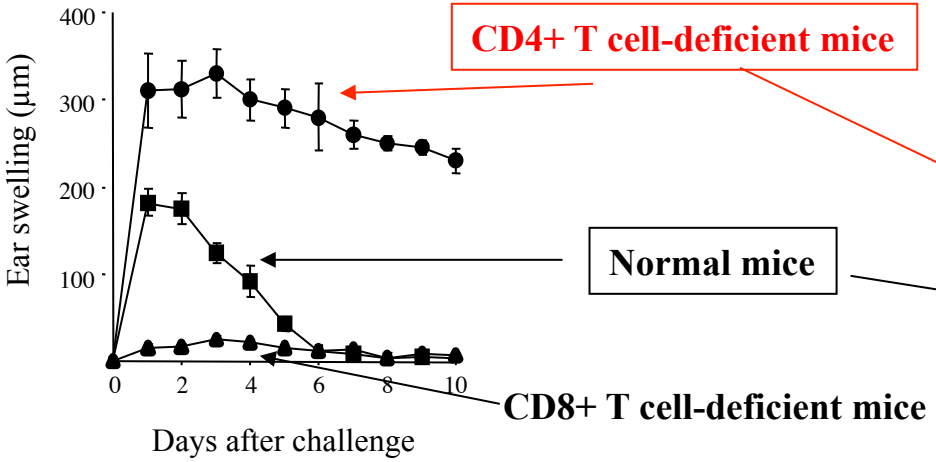
In vivo detection of the sensitizing properties of chemicals

The LLNA-IFNg

Adapt the LLNA protocol to the pathophysiology of ACD = The IFNg-LLNA

Strong haptens
DNFB, Oxazolone

Weak haptens
HCA, Eugenol, Hydroxycitronellal, Dihydrocoumarin, Arnica, Amoxicilline

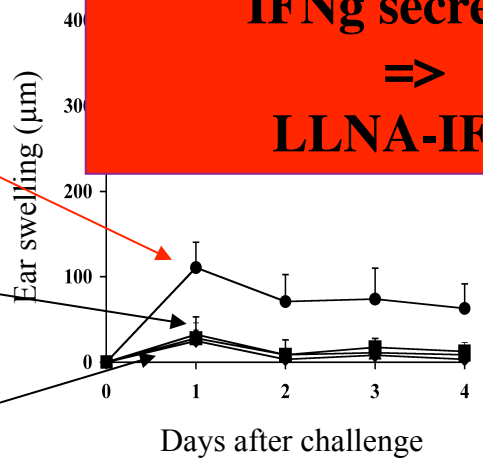


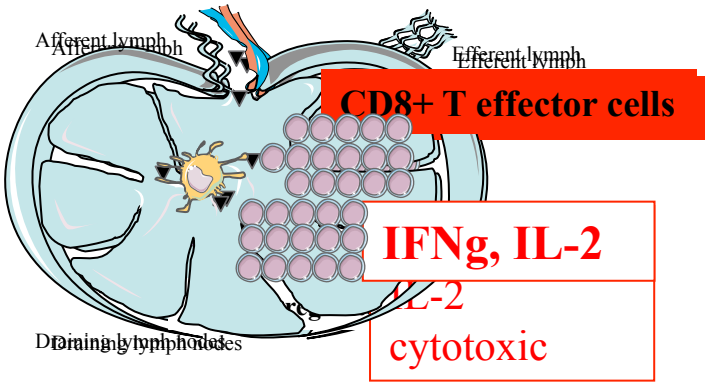
CD4+ T cell-deficient mice

Normal mice

CD8+ T cell-deficient mice

IFNg secretion => LLNA-IFNg

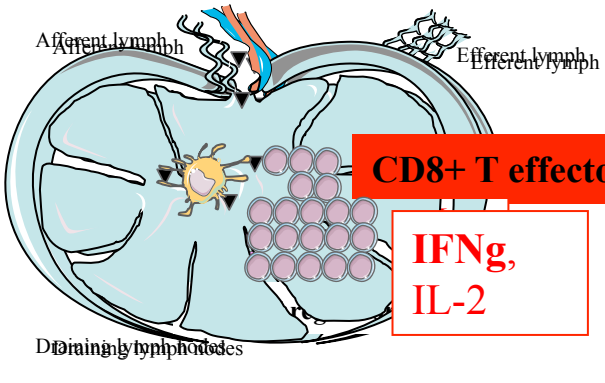




CD8+ T effector cells

IFNg, IL-2

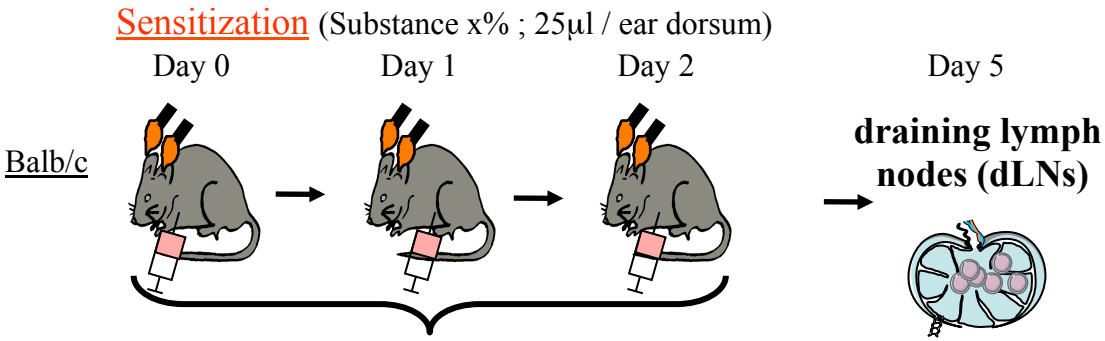
cytotoxic

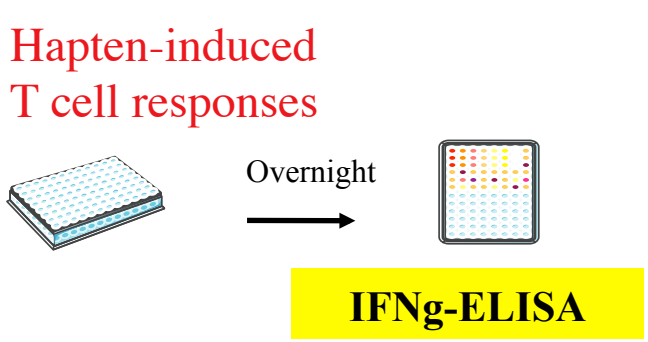


CD8+ T effector cells

IFNg,
IL-2

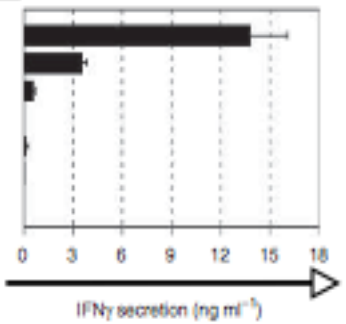
The LLNA-IFNg



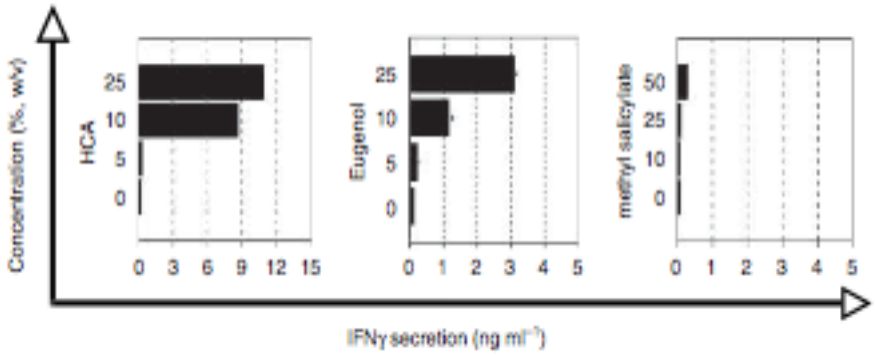


IFNg secretion by LN cells is dramatically increased in the CD4+T-cell-depleted animals upon sensitization

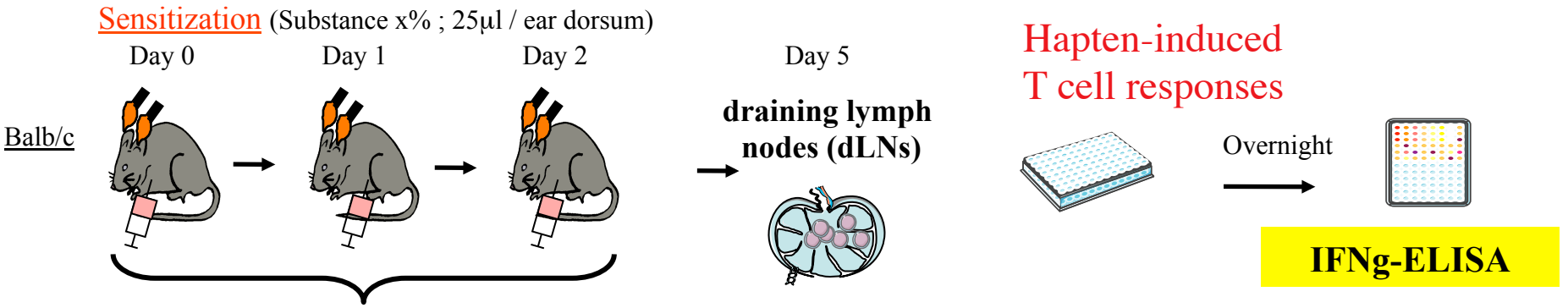
mAb treatment	Sensitization	IFNγ-secretion (ng ml^{-1})	Stimulation indices (SI)
Anti-CD4	Chemical		
+	HCA	13.80	23
+	HDCL	3.585	7
+	A/OO	0.59	–
–	HCA	0.050	6
–	HDCL	0.016	2
–	A/OO	0.008	–



Dose-response induced by haptens but not by non-sensitizers

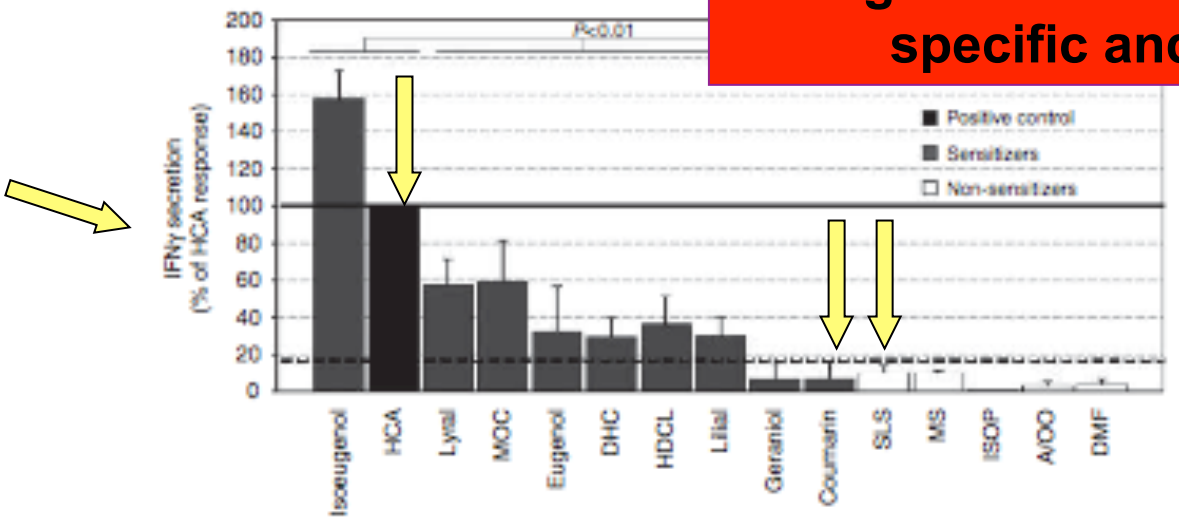


The LLNA-IFNg



The IFNg LLNA detect the sensitizing properties of weak allergens

IFNg-LLNA is non-radioactive, specific and sensitive



Vocanson et al., Skin exposure to weak and moderate contact allergens induces IFNg production by lymph node cells of CD4+T-Cell-Depleted Mice. *J Invest Dermatol. 2009*.
Vocanson et al., The Skin Allergenic Properties of Chemicals May Depend on Contaminants - Evidence from Studies on Coumarin. *Int Arch Allergy Immunol*. 2006
Vocanson et al., Lack of evidence for allergenic properties of coumarin in a mouse model of fragrance allergy. *Contact dermatitis*, 2007

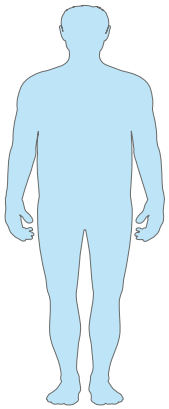
Part 3

In vitro detection of the sensitizing properties of chemicals

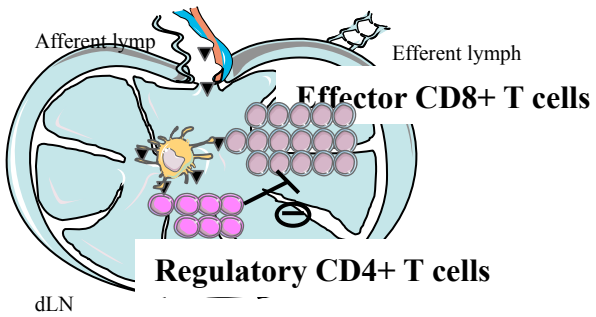
human T Cell Priming Assay (hTCPA)

In vitro alternatives for skin sensitization: hapten-specific T cell activation

Hapten exposure

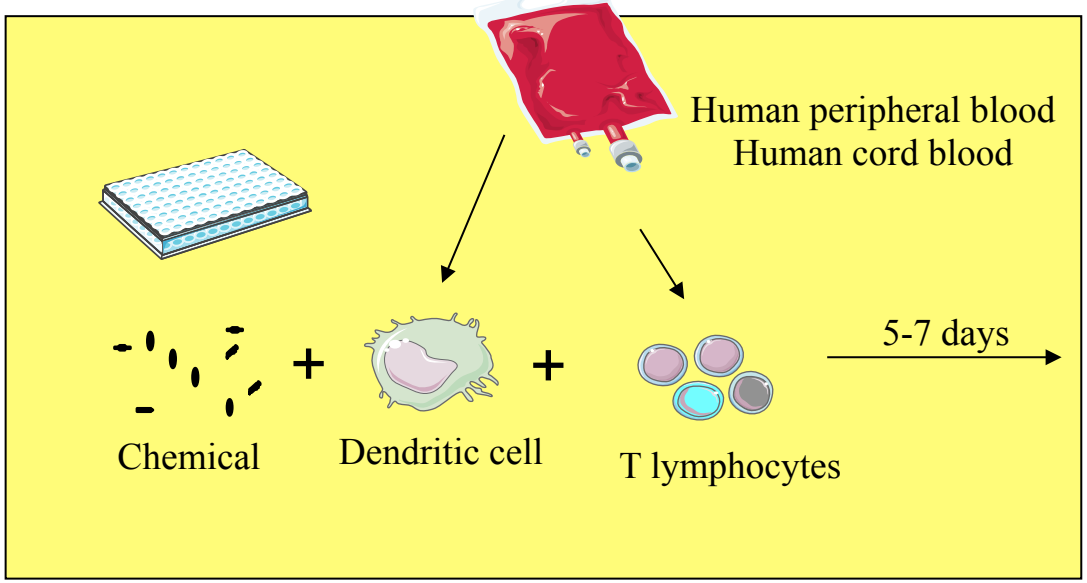


Sensitization: T cell priming



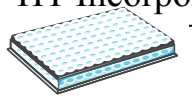
T cell priming assay

Autologous mixed DC-lymphocyte reaction (MDLR)

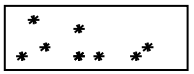


Krasteva et al., Clin Exp Allergy, 1996
Rustemeyer et al., Clin Exp Immunol, 1999
Rougier et al., Toxicology, 2000

^{3}HT Incorporation

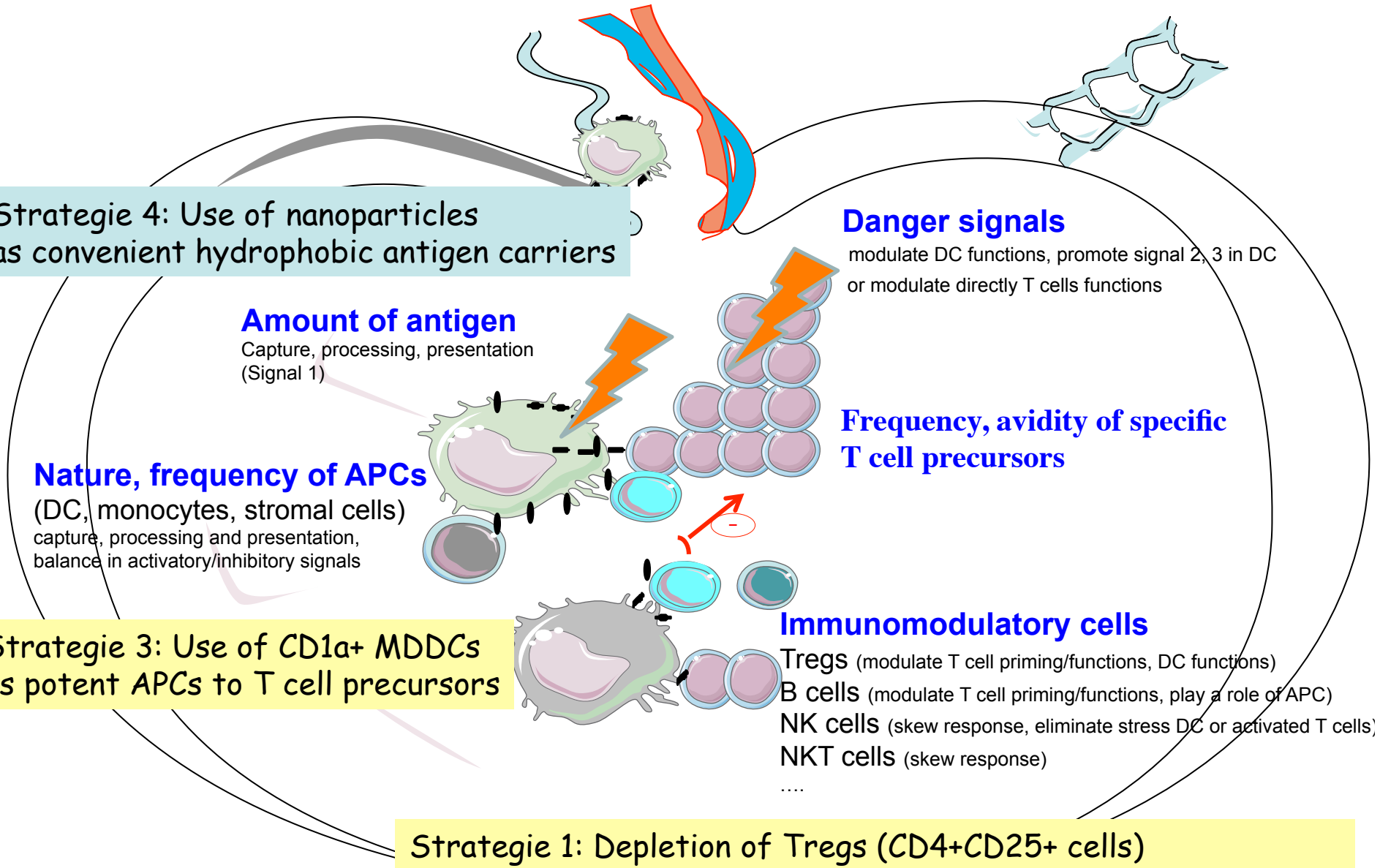


15 hrs



T cell Proliferation

Numerous parameters modulate antigen-specific T cell priming and differentiation in the dLNs



Strategie 4: Use of nanoparticles as convenient hydrophobic antigen carriers

Danger signals
modulate DC functions, promote signal 2, 3 in DC
or modulate directly T cells functions

Amount of antigen
Capture, processing, presentation
(Signal 1)

Frequency, avidity of specific T cell precursors

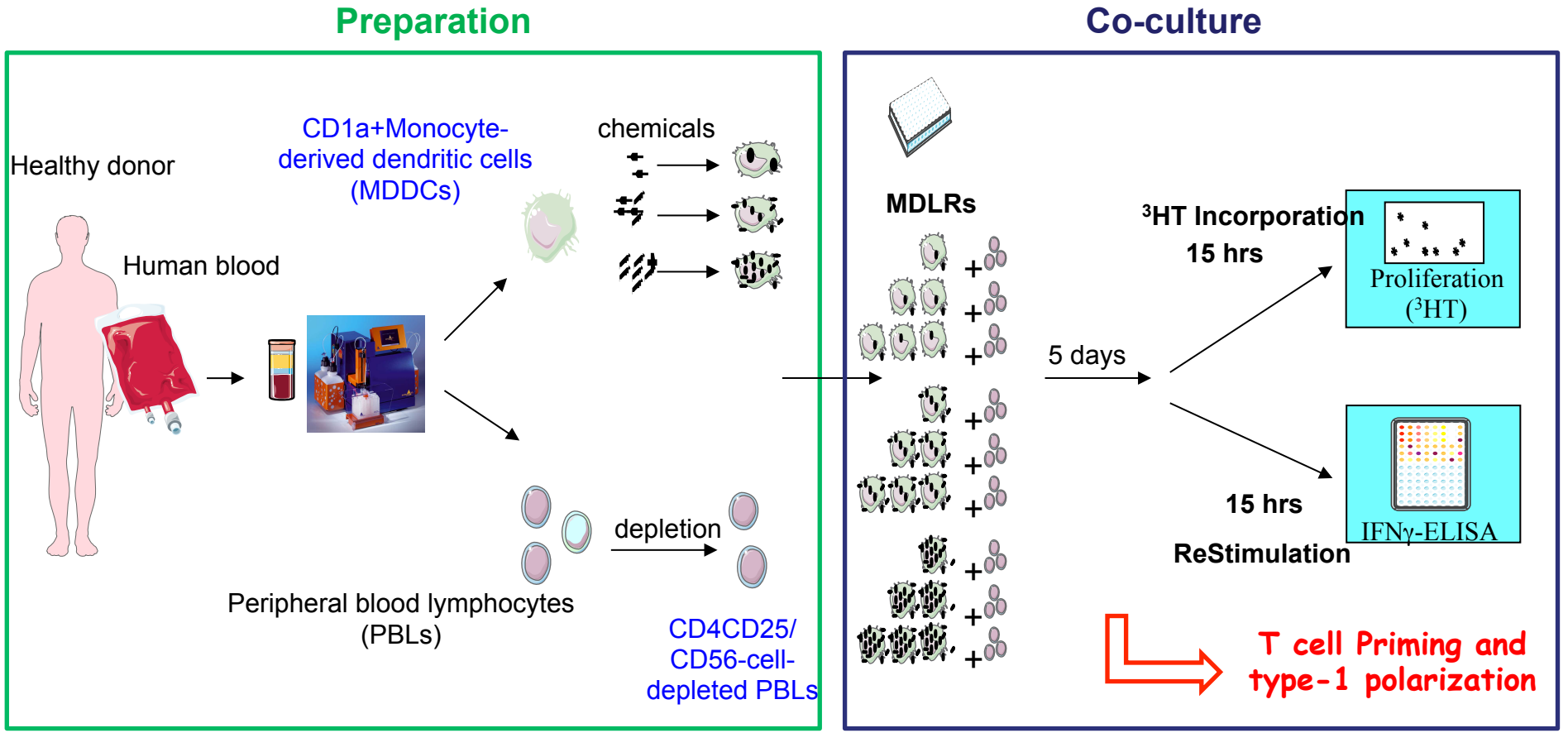
Nature, frequency of APCs
(DC, monocytes, stromal cells)
capture, processing and presentation,
balance in activatory/inhibitory signals

Strategie 3: Use of CD1a+ MDDCs as potent APCs to T cell precursors

Immunomodulatory cells
Tregs (modulate T cell priming/functions, DC functions)
B cells (modulate T cell priming/functions, play a role of APC)
NK cells (skew response, eliminate stress DC or activated T cells)
NKT cells (skew response)
....

Strategie 1: Depletion of Tregs (CD4+CD25+ cells)
Strategie 2: Depletion of immunomodulatory cells (CD56+ cells)

hTCPA Protocol



Estimation of the sensitizing potency of chemicals

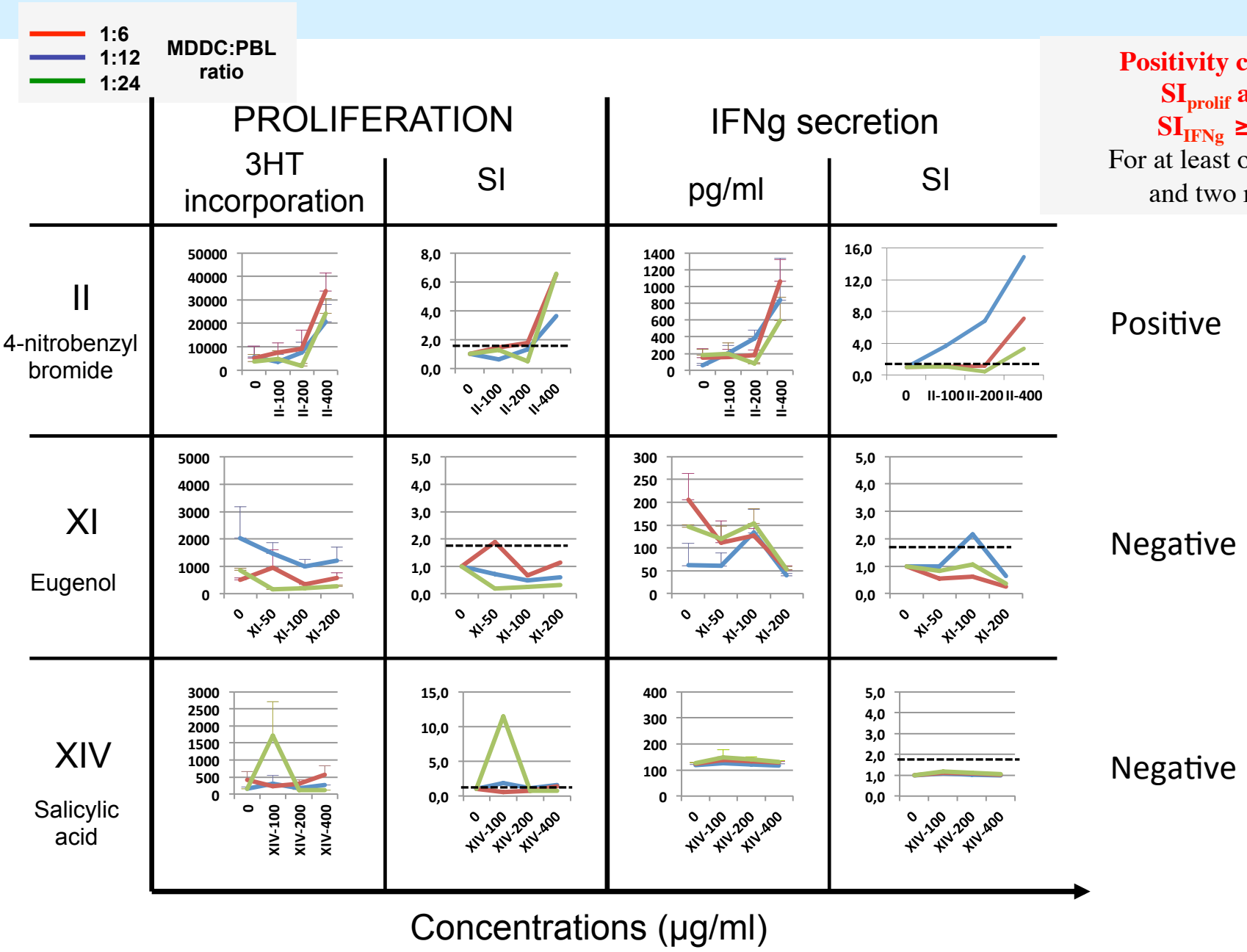
- A test chemical is positive in the hTCPA =
  - Both robust proliferation and IFNg secretion, that is SI_{prolif} and a $SI_{IFNg} \geq 1.8$
  - for at least one dose and two MDDC/PBL ratios
- 1 positive hTCPA experiment = sufficient to declare a chemical as a sensitizer

hTCPA - Blinded study – Decoding

- 16 blinded compounds were tested on at least 3 different donors
- Decoding was provided by Cosmetics Europe (**12 reference sensitizers**, **4 non-sensitizers**)

Chemicals	Doses (mg/ml)	Name
Compound I	50, 100, 200, 400	4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one (Oxazolone)
Compound II	100, 200, 400	4-Nitrobenzyl bromide
Compound III	50, 100, 200	1-Chloro-2,4-dinitrobenzene (DNCB)
Compound IV	25, 50, 100	Methyldibromoglutaronitrile (MDGN)
Compound V	100, 200, 400	Glyoxal solution
Compound VI	200, 400, 800	2-Mercaptobenzothiazole (MBT
Compound VII	100, 200, 400	Cinnamaldehyde
Compound VIII	200, 400, 800	Tetramethylthiuram disulfide (TMTD)
Compound IX	50, 100, 200	Phenylenediamine (PPD)
Compound X	100, 200, 400	Isoeugenol
Compound XI	50, 100, 200	Eugenol
Compound XII	200, 400, 800	Cinnamyl alcohol
Compound XIII	25, 50, 100	Glycerol
Compound XIV	100, 200, 400	Salicylic acid
Compound XV	50, 100, 200, 400	Lactic acid
Compound XVI	12,5, 25, 50	Sodium dodecyl sulfate (SLS)

hTCPA - Blinded study – Examples of results



Positivity criteria
SI_{prolif} and
$SI_{IFNg} \geq 1.8$
For at least one dose and two ratio

Positive

Negative

Negative

hTCPA - Blinded study – The hTCPA is sensitive and specific

- 16 blinded compounds were tested on at least 3 different donors
- Decoding was provided by Cosmetics Europe (12 reference sensitizers, 4 non-sensitizers)

Chemicals	Name	Number of positive hTCPA responses
Compound I	4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one (Oxazolone)	**0/5**
Compound II	4-Nitrobenzyl bromide	**2/3**
Compound III	1-Chloro-2,4-dinitrobenzene (DNCB)	**2/4**
Compound IV	Methyldibromoglutaronitrile (MDGN)	**1/3**
Compound V	Glyoxal solution	**0/4**
Compound VI	2-Mercaptobenzothiazole (MBT	**1/3**
Compound VII	Cinnamaldehyde	**1/3**
Compound VIII	Tetramethylthiuram disulfide (TMTD)	**2/3**
Compound IX	Phenylenediamine (PPD)	**0/5**
Compound X	Isoeugenol	**1/4**
Compound XI	Eugenol	**0/4**
Compound XII	Cinnamyl alcohol	**1/3**
Compound XIII	Glycerol	**0/4**
Compound XIV	Salicylic acid	**0/3**
Compound XV	Lactic acid	**0/3**
Compound XVI	Sodium dodecyl sulfate (SLS)	**0/3**

8 /12 => positive results

Sensitivity =67%

False negative

0 / 4 => negative results

Specificity =100%

hTCPA – Major issues addressed by the results

1. Can we declare a chemical as a sensitizer, when only one positive response was obtained with one tested donor?

   Inconsistency in donor response was expected (Individual polymorphism in T cell repertoire, or in MDDC response to electrophilic stress)

2. Can we imagine that some donors are more prone to respond to hapten stimulation?

   Positive response in a limited number of donors

3. We recommend to test a chemical on 3 to 5 different donors

4. What hypothesis for the negative results recorded with some reference sensitizers?
   - Solubility (Oxazolone)
   - Glyoxal always negative
   - Oxydation (para-phenylediamine) issues? (Our PPD = sensitizer)

5. What recommendations to improve hTCPA sensitivity ?
   - Future hTCPA decision tree -> oxydation step?, use of nanoparticles?

   - To refine read-outs and analyse complementary markers
   - To introduce a secondary restimulation step to accurately detect Ag-specificity

   => ongoing collaborative work with the group of S.Martin, Freiburg

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“Immunology of skin allergy and vaccination”

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