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In-vitro quantitation of sensitizing potential

Definitions (OECD guideline 431)

corrosive

means…any substance which in contact with living tissue will cause **destruction** of tissue **by chemical action**…but shall not refer to action on inanimate surfaces

irritant

means any substance not corrosive which on immediate, prolonged, or repeated contact with normal living tissue will induce a **local inflammatory reaction**

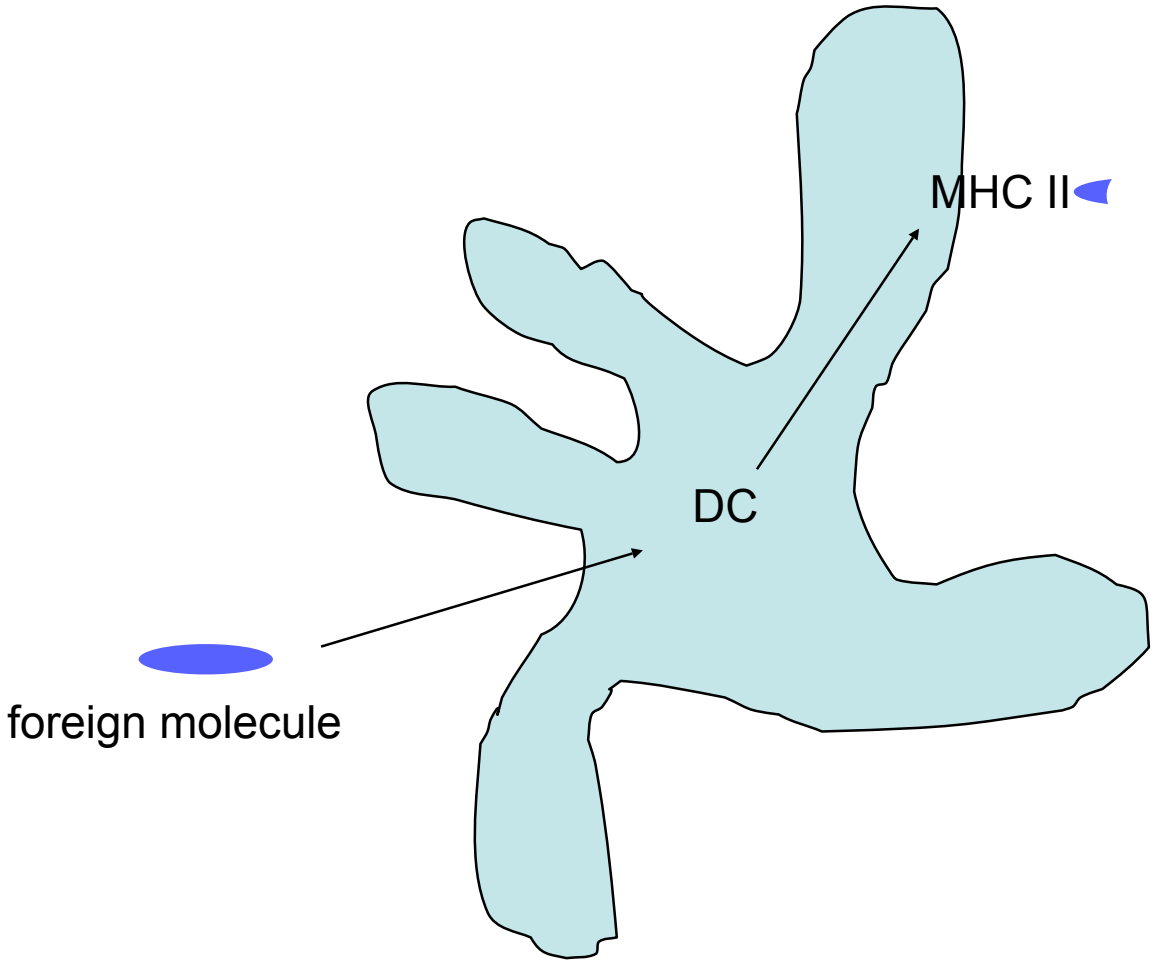
sensitizer

means a substance that will cause on normal living tissue through an **allergic process** a **hypersensitivity** which becomes **evident on reapplication** of the same substance

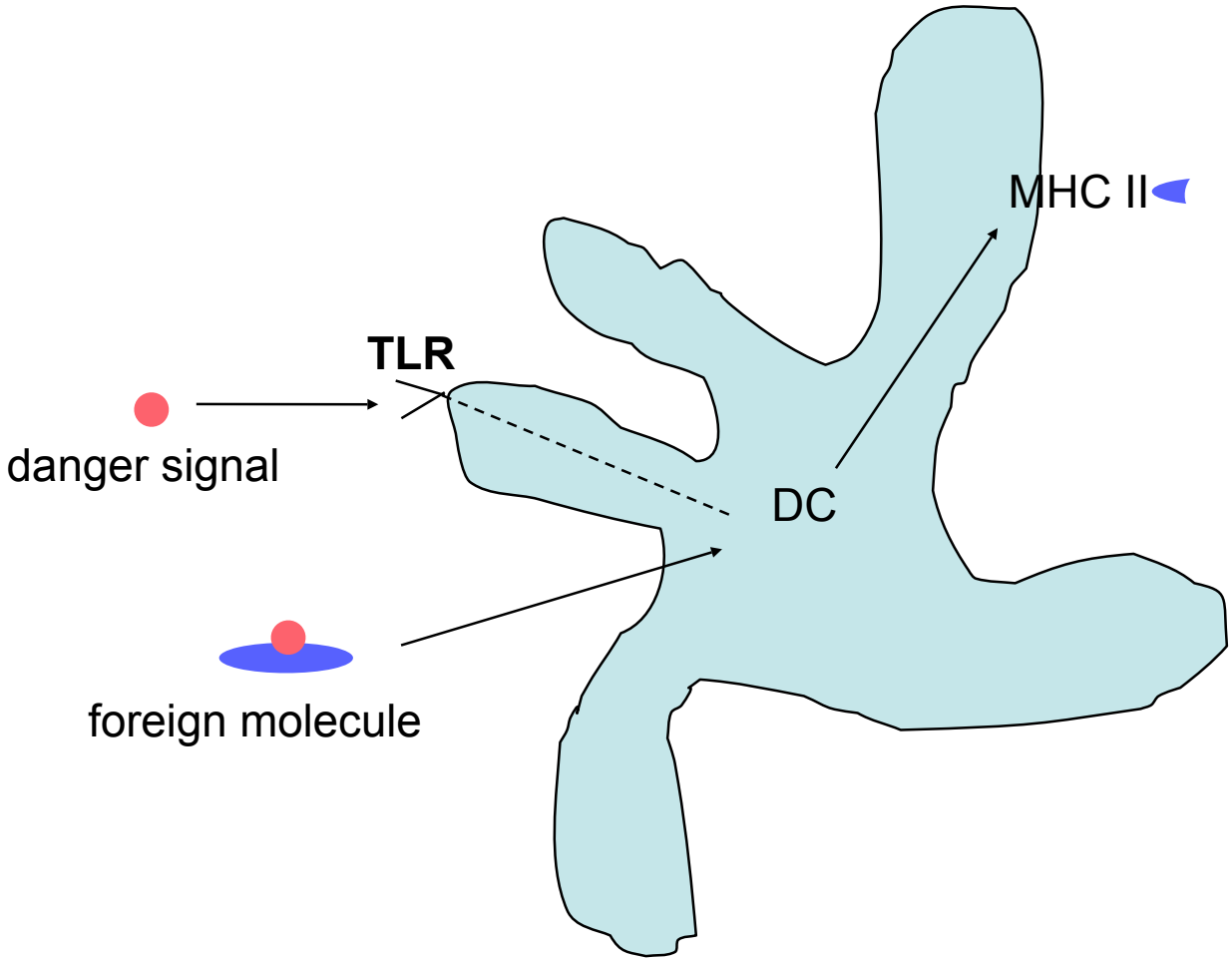
→ activation of the specific immune system

→ contact allergy

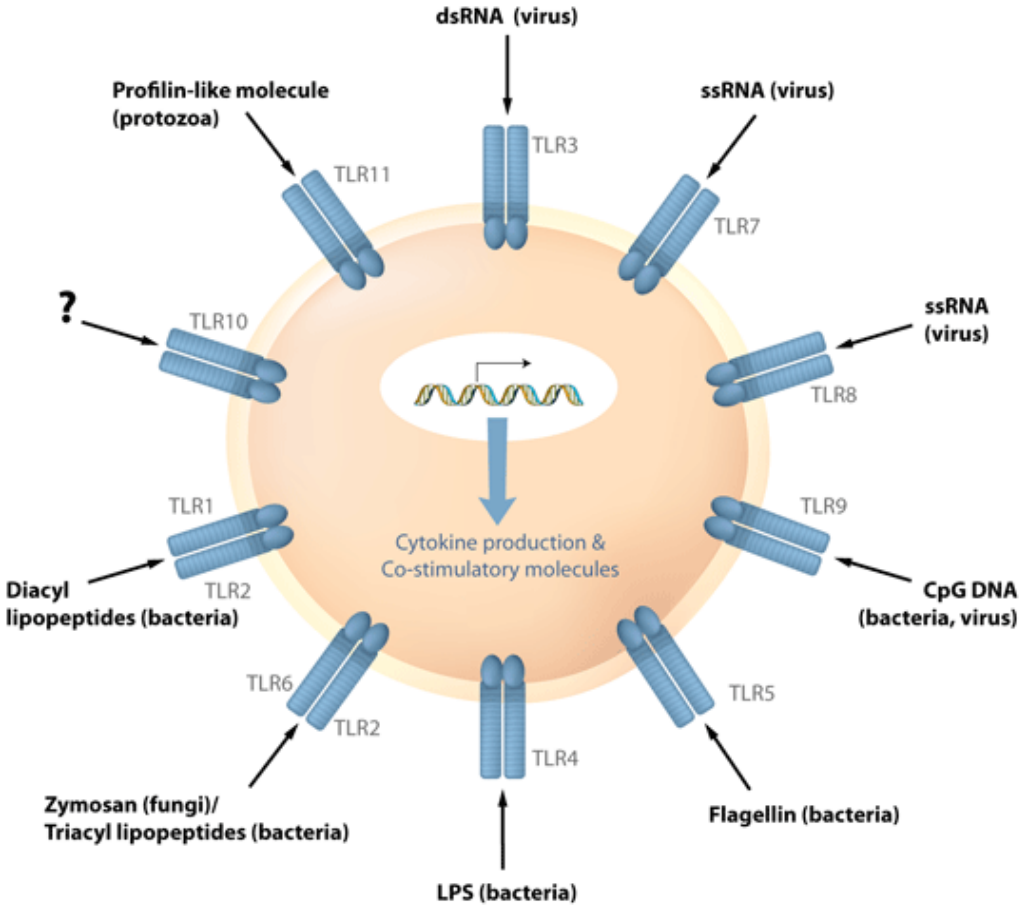
Activation of the specific immune system by **Dendritic cells**,
the watchdogs of the immune system



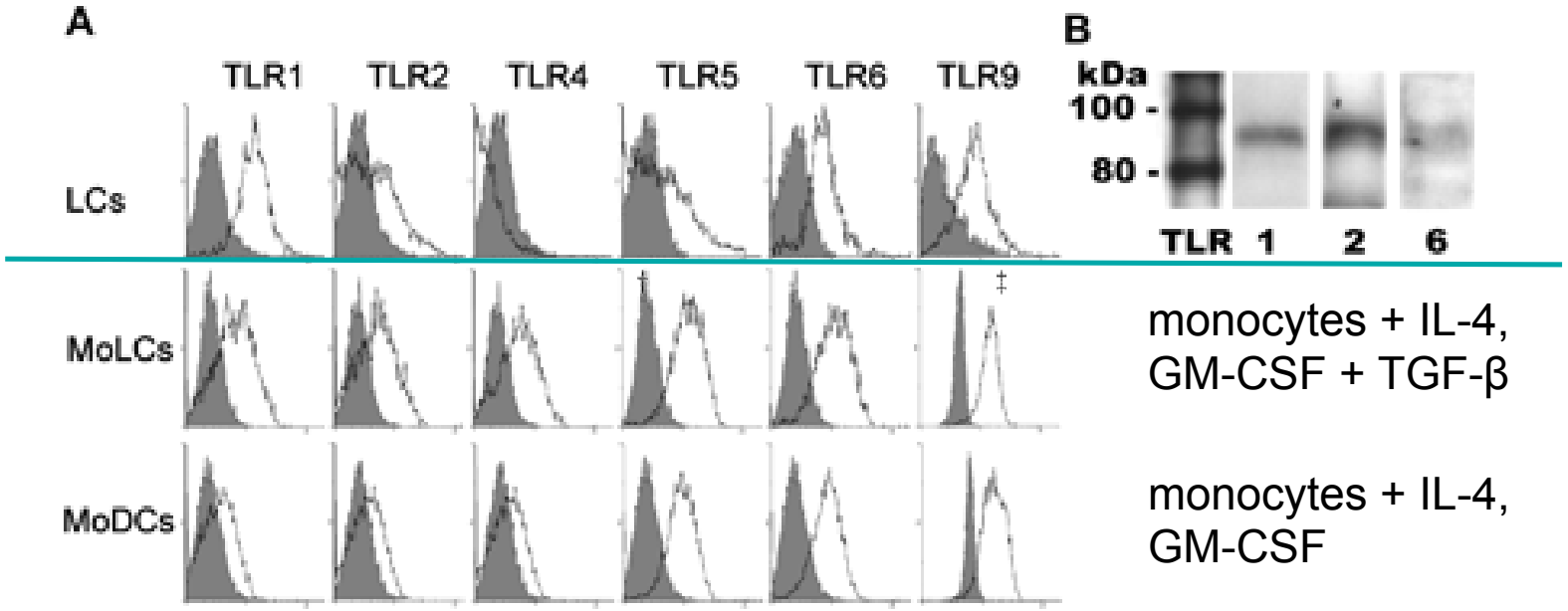
Activation needs **danger signals**: pathogen associated molecules



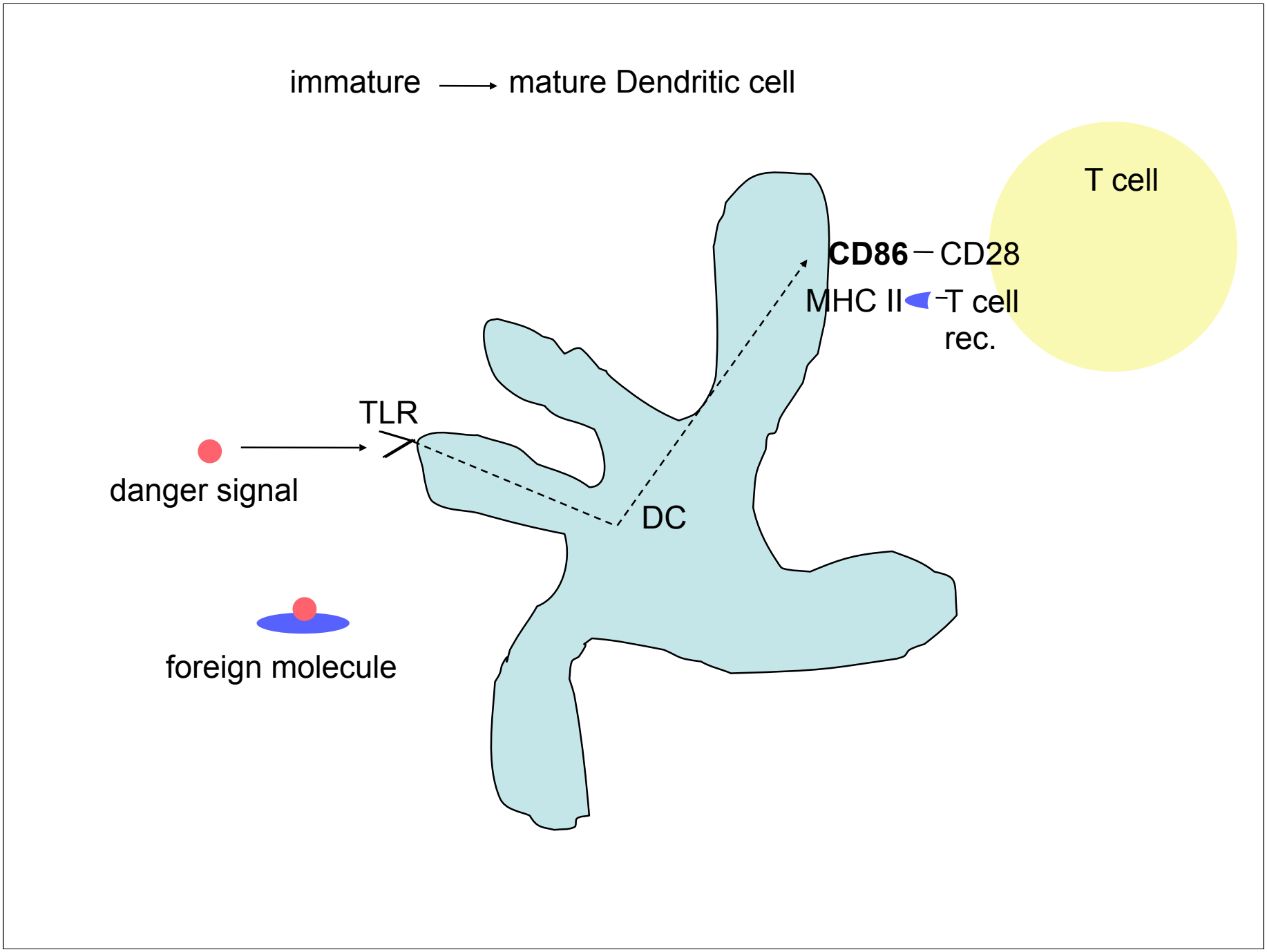
TLR,Toll-like receptors: evolved to detect pathogen-associated molecules



Equipment of Langerhans cells and of monocyte-derived Dendritic cells with **TLRs**



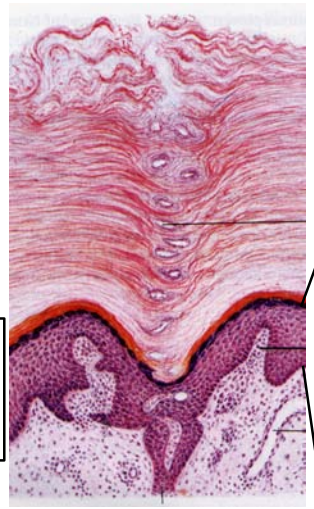
From: Peiser M, Koeck J, Kirschning CJ, Wittig B, Wanner R (2008) J. Leukoc. Biol. 83:1118-27.


immature → mature Dendritic cell
T cell
CD86 – CD28
MHC II – T cell rec.
TLR
danger signal
DC
foreign molecule

Penetration

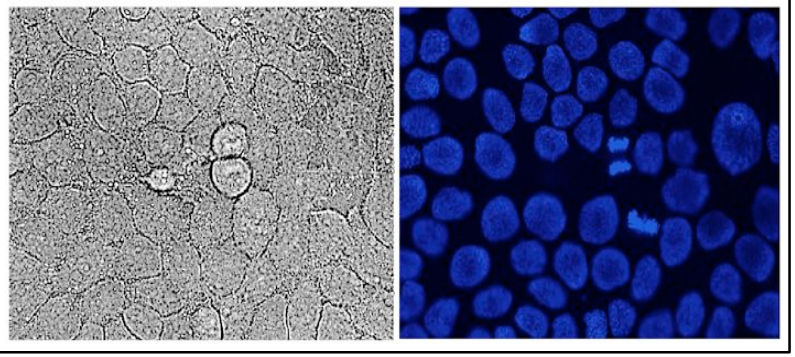
Sensitization

Allergen



I. Access to the viable epidermis

II. Metabolism and combination with a protein by **keratinocytes**

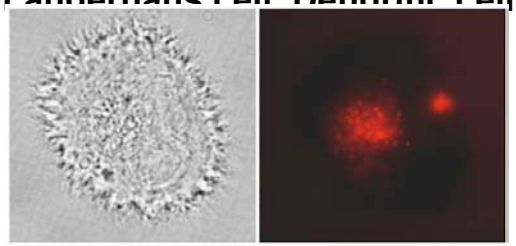


Foreign immunogenic molecule

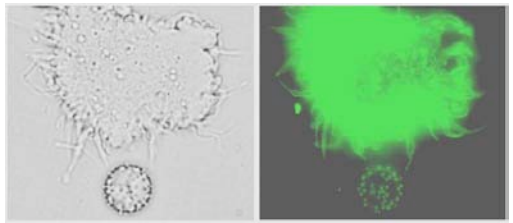
danger signals

local trauma

III. Uptake and presentation on **Langerhans cell, Dendritic cell**



IV. Maturation to an **Antigen-presenting cell**



An in-vitro assay for sensitization should integrate
Dendritic cells **and** keratinocytes

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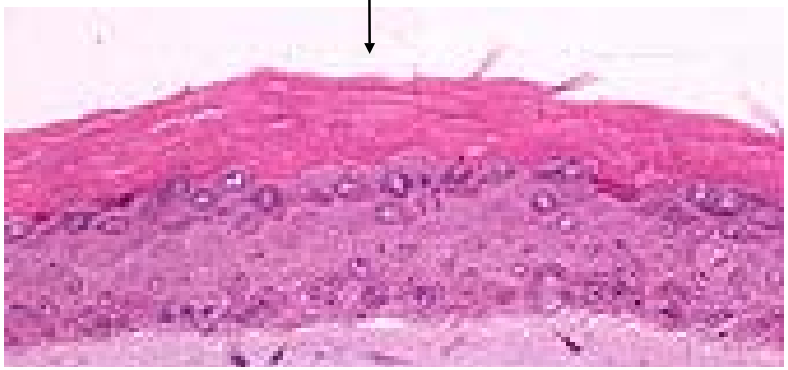
In-vitro:

3-dimensional skin equivalent

keratinocytes,
fibroblasts,
melanocytes

Testing of corrosive and irritant potential



Read-out: viability assays (as MTT)

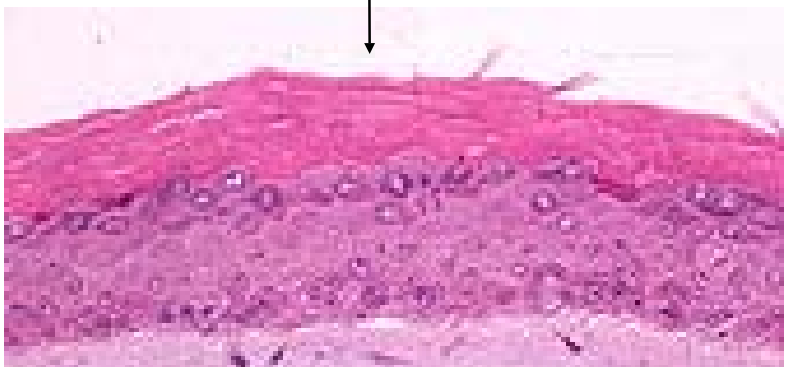
In-vitro:

3-dimensional skin equivants

keratinocytes, fibroblasts, melanocytes

+ Dendritic cells

Testing of sensitizing potential



Read-out: activation of Dendritic cells

as CD86-upregulation or specific cytokine secretion

Problem:

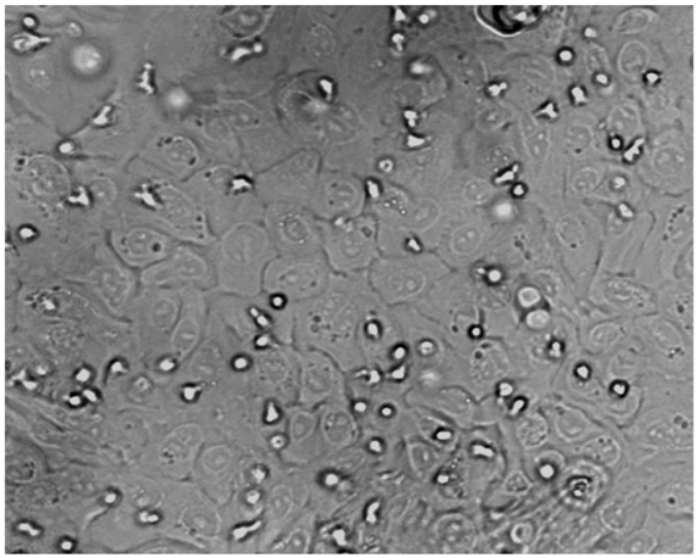
Dendritic cells do not integrate steadily into 3D-cultures

In vivo, DC are also not fixed steadily to the keratinocytes

They do not wait for an allergen to drop by. Rather, they move around and scan actively for foreign substances

More natural model: a loose-fit coculture of keratinocytes and Dendritic cells

LCSA® = loose-fit coculture-based sensitization assay



Loose-fit coculture of human non-differentiating keratinocytes and of human monocytes

+ cytokine cocktail
IL-4, GM-CSF, TGF-β

Activated keratinocytes
+ DC-related cells

→ Secrete the matrix-metalloproteinase MMP-9

→ are $CD1c^{dim}$, $CD1a^{-}$, $Langerin^{-}$

The LCSA can be conducted within in 1 week:

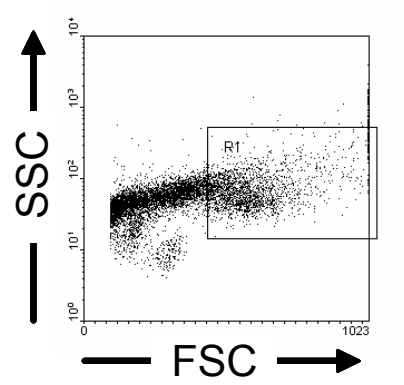
Monday	seeding of cryopreserved keratinocytes
	adherence after 2 h
	seeding of cryopreserved PBMC (peripheral blood mononuclear cells)
	after 1.5 h rinsing off the floating PBMC cells
	addition of cytokines
Wednesday	application of test substances
Friday	harvest of floating cells
	antibody staining
	FACS analysis (CD86)

Application of TNBS to the LCSA®

CD14-isolated monocytes

IL-4, GM-CSF, TGF-β treated

cultured alone

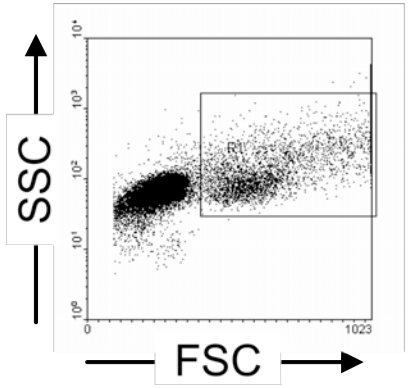


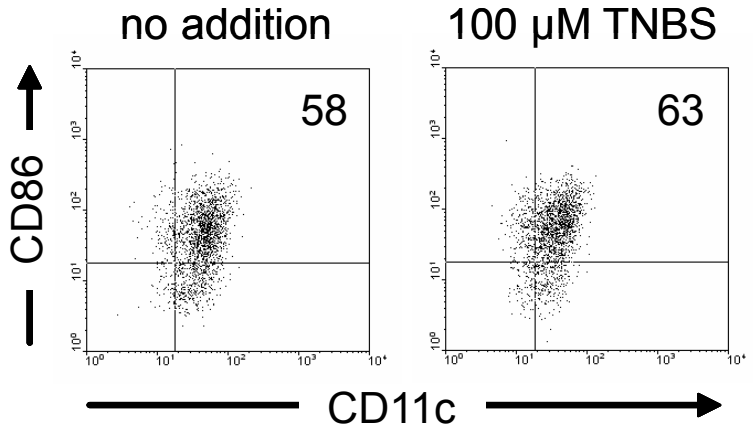
CD14-isolated monocytes

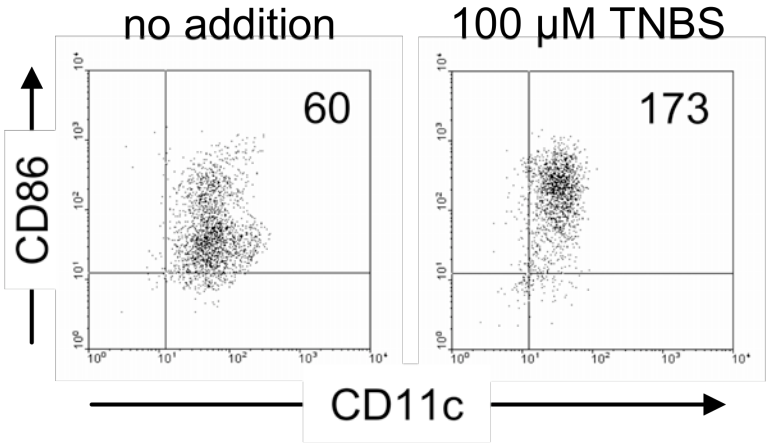
IL-4, GM-CSF, TGF-β treated

during

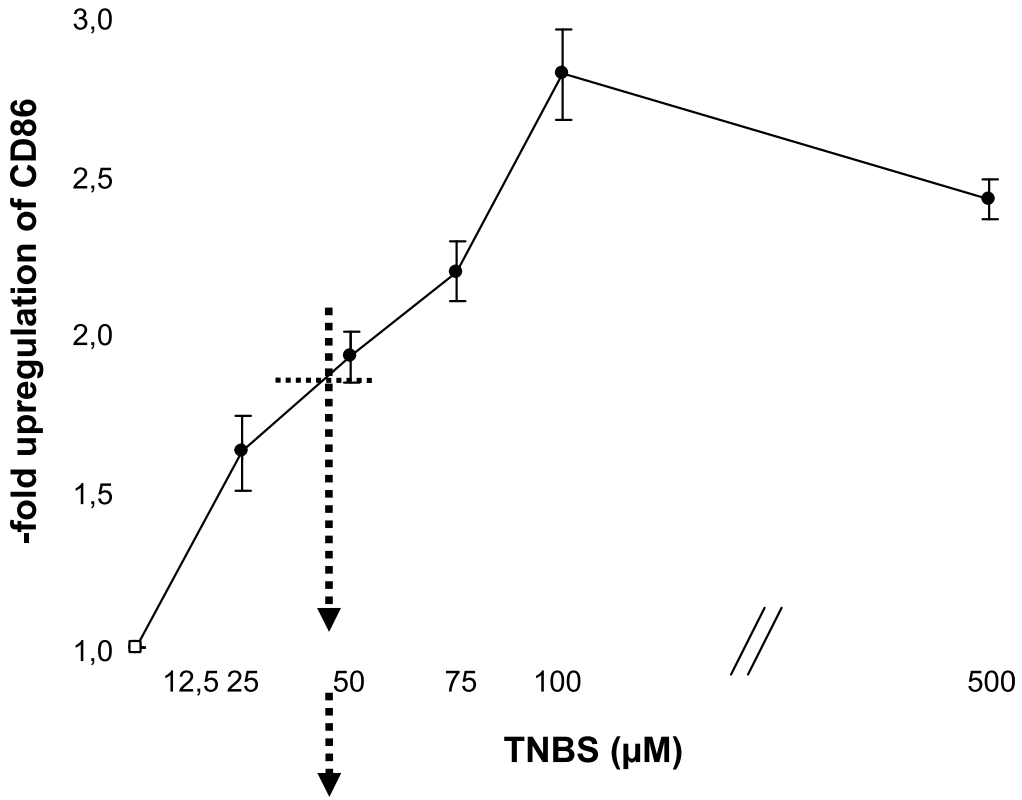
coculture with KC





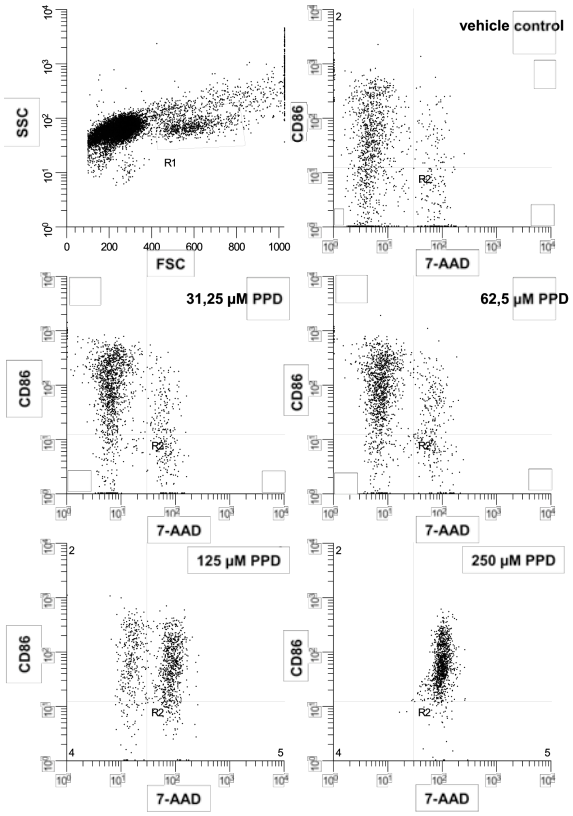


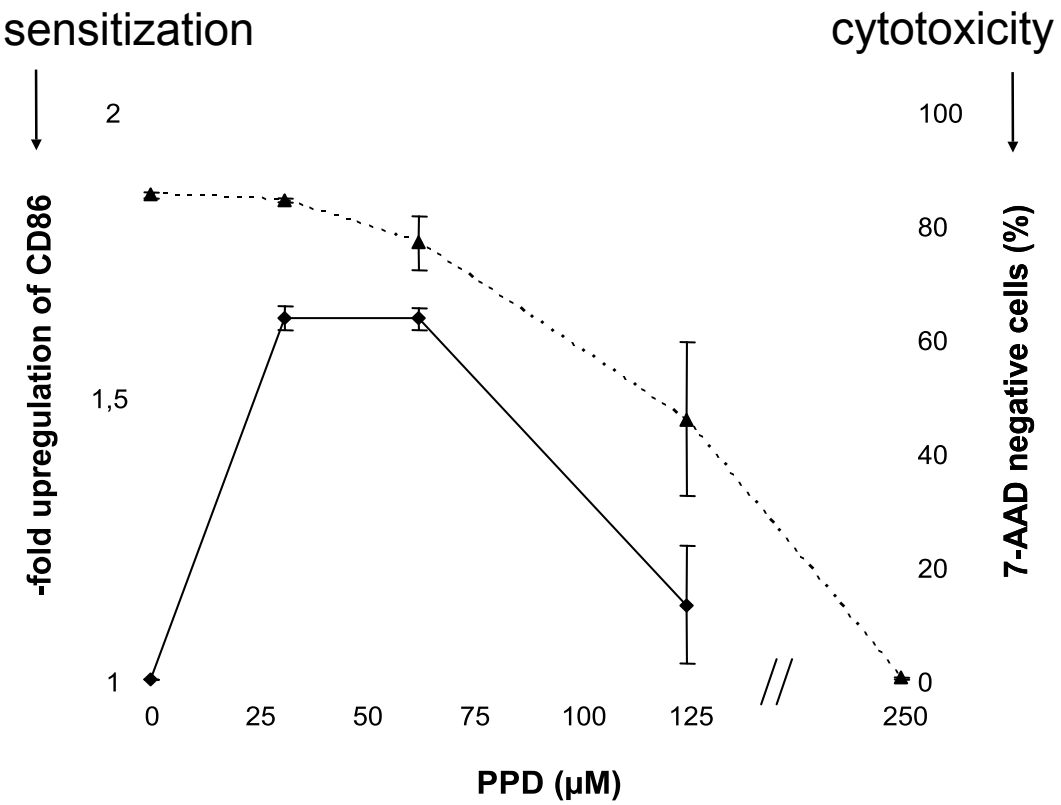
LCSA: concentration- response relationship to Trinitrobenzene sulfonic acid (TNBS), a strong contact allergen

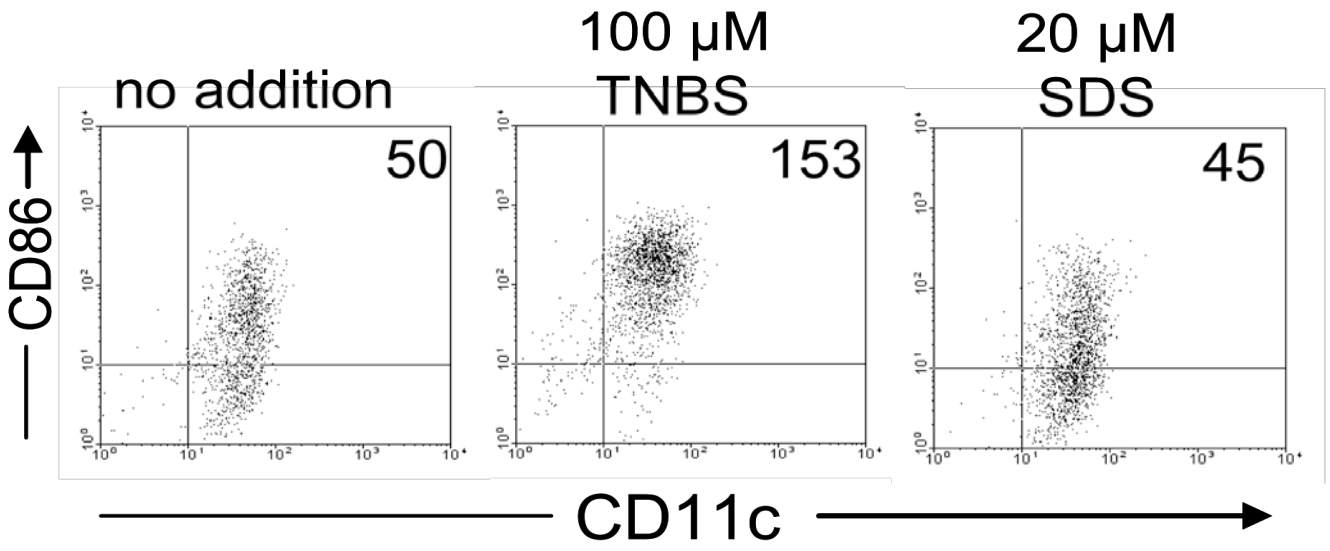


unprecedented sensitivity: half-maximal increase at 40 µM

High sensitivity allows testing at concentrations without general cytotoxicity: discrimination between allergic and irritant potential





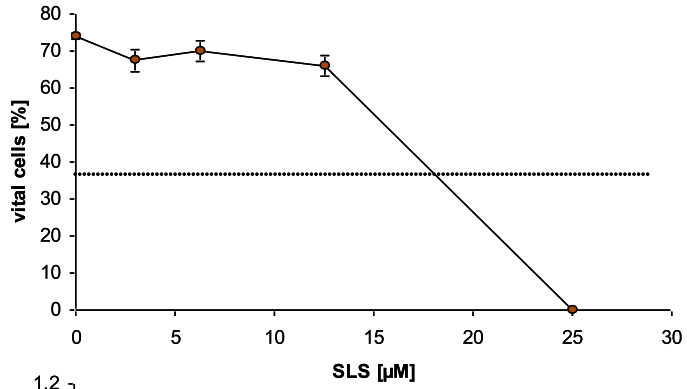


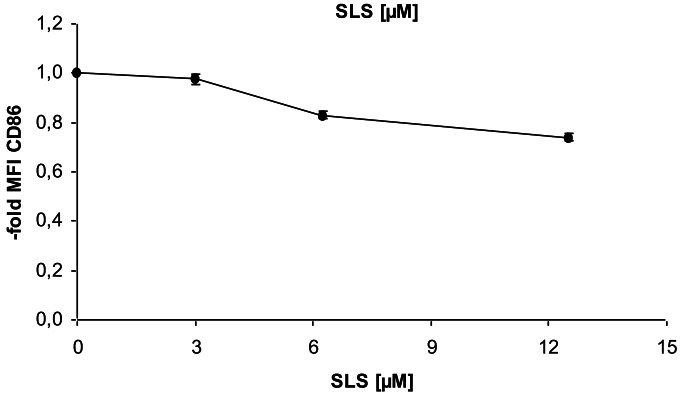
The irritant SDS is negative in LCSA

↕

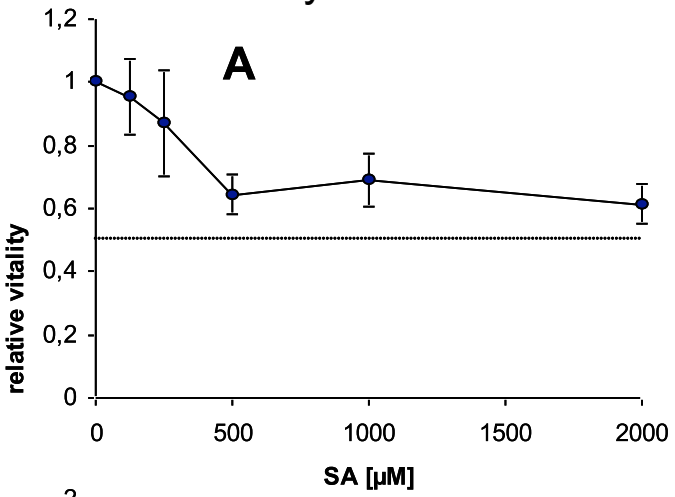
false-positive in LLNA

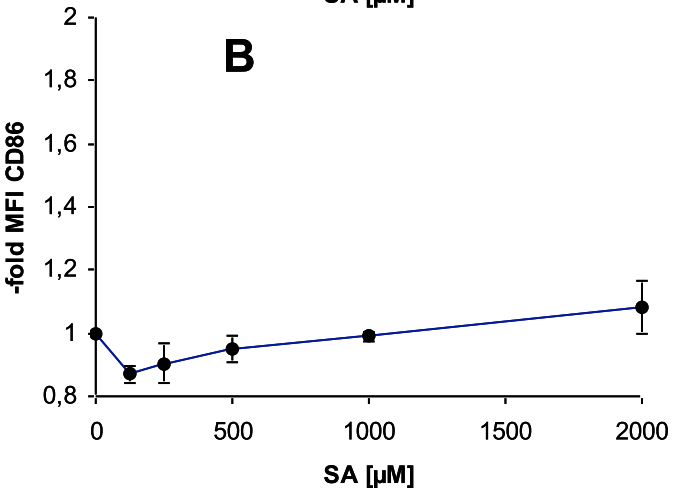
Sodium lauryl sulfate

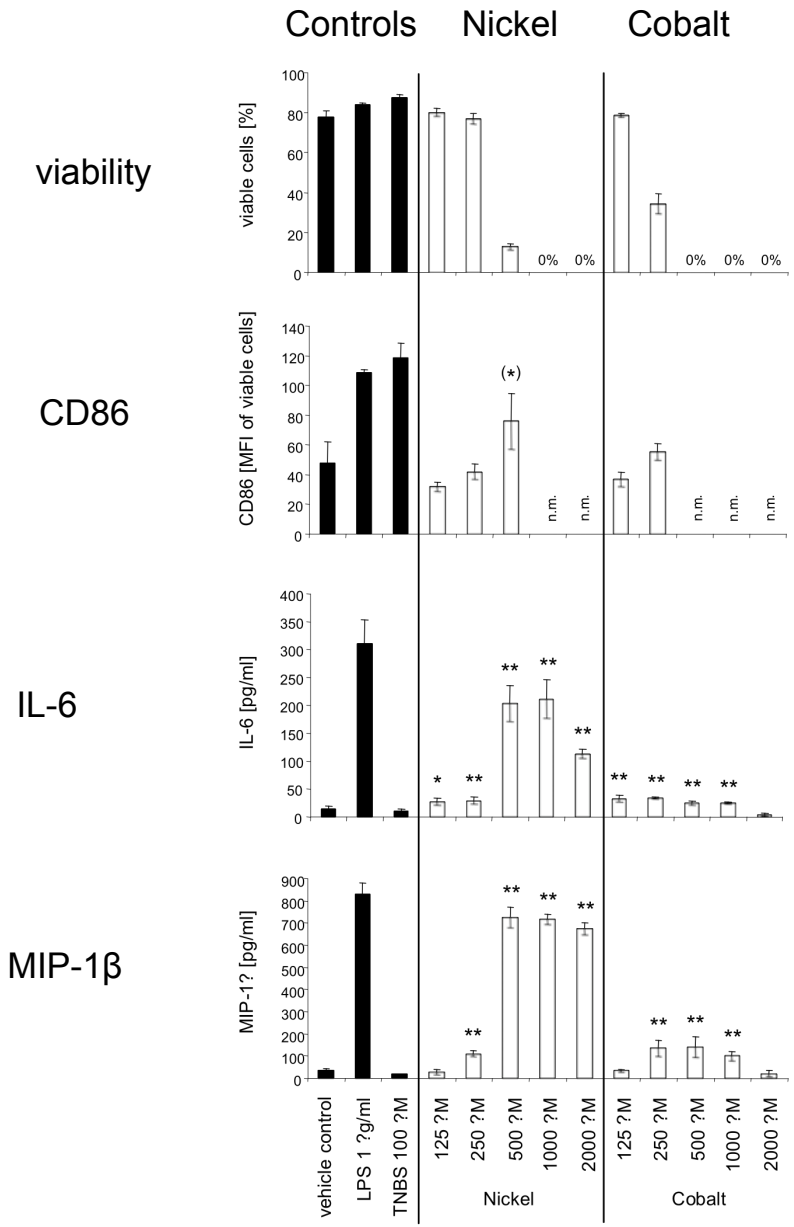


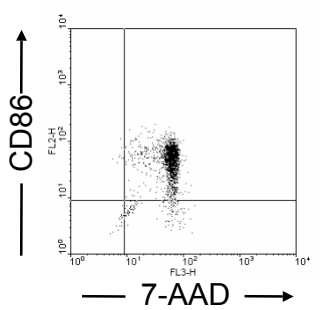


Salicylic acid






Controls
Nickel
Cobalt
viability
CD86
IL-6
MIP-1β
viable cells [%]
CD86 [MFI of viable cells]
IL-6 [pg/ml]
MIP-1? [pg/ml]
0%
n.m.
(*)
*
**
vehicle control
LPS 1 ?g/ml
TNBS 100 ?M
125 ?M
250 ?M
500 ?M
1000 ?M
2000 ?M
Nickel
Cobalt


CD86
7-AAD
FL3-H

Substance	½-max. increase in MFI CD86 [µM] by LCSA	potency category as categorized by LCSA	potency category as categorized by LLNA	LLNA, EC3%
Group A, accordance between LCSA and LLNA				
Cinnamal (CA)	60	moderate	moderate	3,10
Isoeugenol	140	moderate	moderate	1,80
PTD	16	strong	strong	0,31
TNBS	40	strong	strong	0,30
DNCB	5	strong	strong	0,04
PPD (Brand. Base)	16	strong	strong	0,03
salicylic acid (SA)	negative	negative	negative	
phenol	negative	negative	negative	
TMA	negative	*(negative)*	*(negative)*	
Group B, no accordance between LCSA and LLNA				
HCA (Hexylcinnamaldehyde	3**	*(strong)*	moderate	8,40
Sodium lauryl sulfate (SLS)	negative	negative	*(moderate)*	3,7
MDI	negative	*(negative)*	positiv	
Nickel		positive*	(*negative)*	

LCSA, performance

50 different cell-donor pairs	no donor-dependent variance detectable no problem with genetic instability no ethical or logistical problems cells cryopreservable, no use of serum
22 different substances	discrimination between allergens and irritants or between sensitizing and cytotoxic/irritative concentration of a substance isoeugenol (prohapten) and hexylcinnamaldehyde (HCA) detectable discrimination between allergens and the pathogenic molecule LPS discrimination between metal-allergens and small-sized molecules

Coworkers

Maximilian Schreiner

Anna Sonnenburg

Matthias Peiser

Cooperations

Ralf Stahlmann

Torsten Zuberbier

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