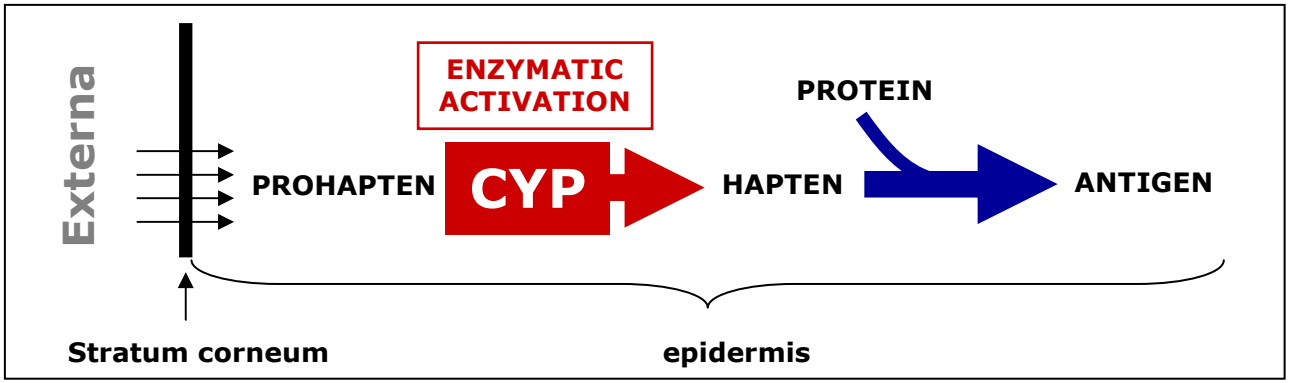
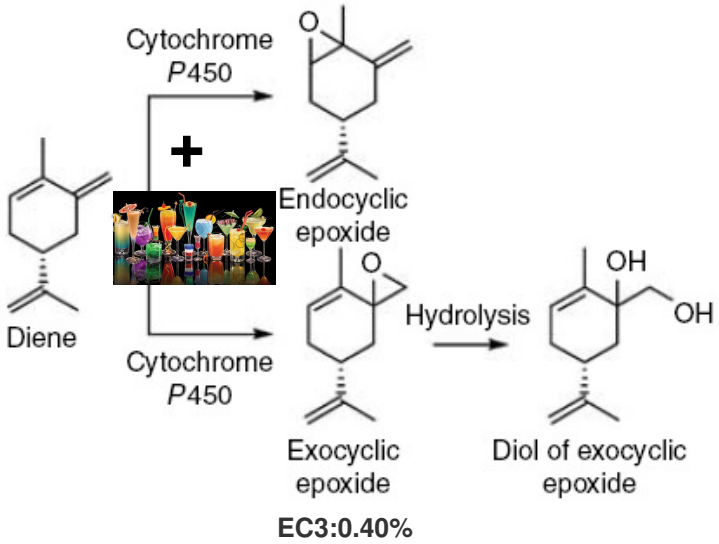
Sherlock Holmes goes molecular: Allergische Kontaktdermatitis von Patch Test zu Prohaptenen (II)

Hans F Merk

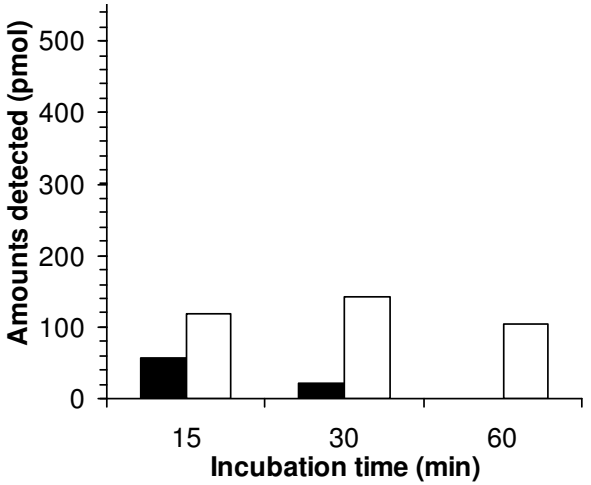
Dept. of Dermatology & Allergology – Univ.-Hospitals – RWTH Aachen

Activation of prohaptens is mediated by CYPs expressed in skin cells





Chem. Res. Toxicol 18:308-16,2005
J Invest Dermatol, 127: 1145–1153, 2007



Detection of a **hapten** (diol of exocyclic epoxide: □) after incubation of the model-**prohapten** ((5R)-5-isopropenyl-2-methyl-1-methylene-2-cyclohexene) with a skin specific **rhCYP-Cocktail**

Human moDC in vitro assay

Characterization of the Sensitizing Potential of Chemicals by *In Vitro* Analysis of Dendritic Cell Activation and Skin Penetration

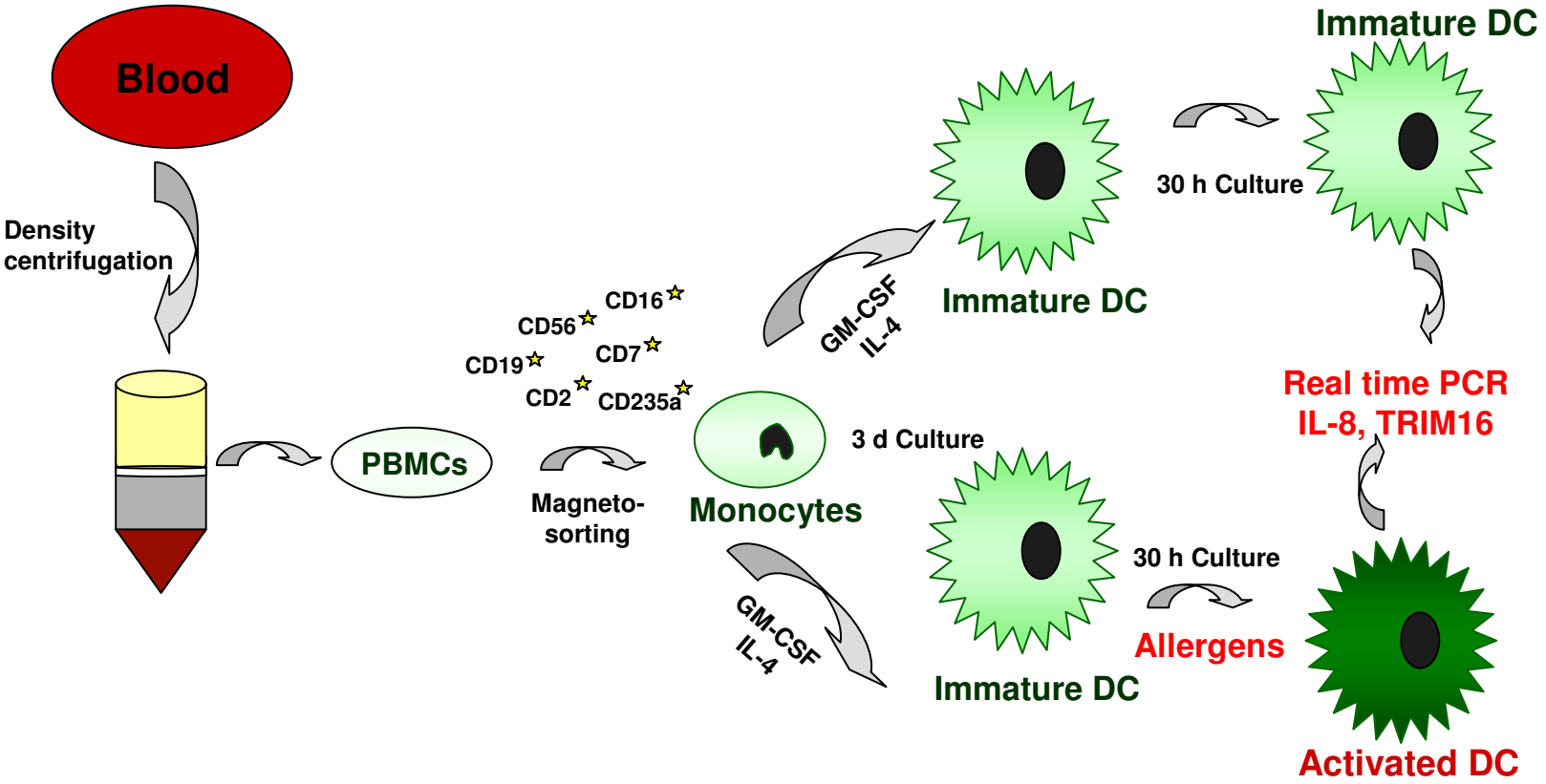
Pierre Aeby,* Christoph Wyss,* Heinz Beck,* Peter Griem,§ Heike Scheffler,† and Carsten Goebel†

J Invest Dermatol 122:1154–1164, 2004

High-Resolution Transcriptional Profiling of Chemical-Stimulated Dendritic Cells Identifies Immunogenic Contact Allergens, but Not Prohaptens

H. Ott[a] T. Wiederholt[a] M. Andresen Bergström[b] R. Heise[a] C. Skazik[a] K. Czaja[a] Y. Marquardt[a] A.-T. Karlberg[b] H.-F. Merk[a] J.M. Baron[a]

Skin Pharmacol Physiol 2010;23:213–224



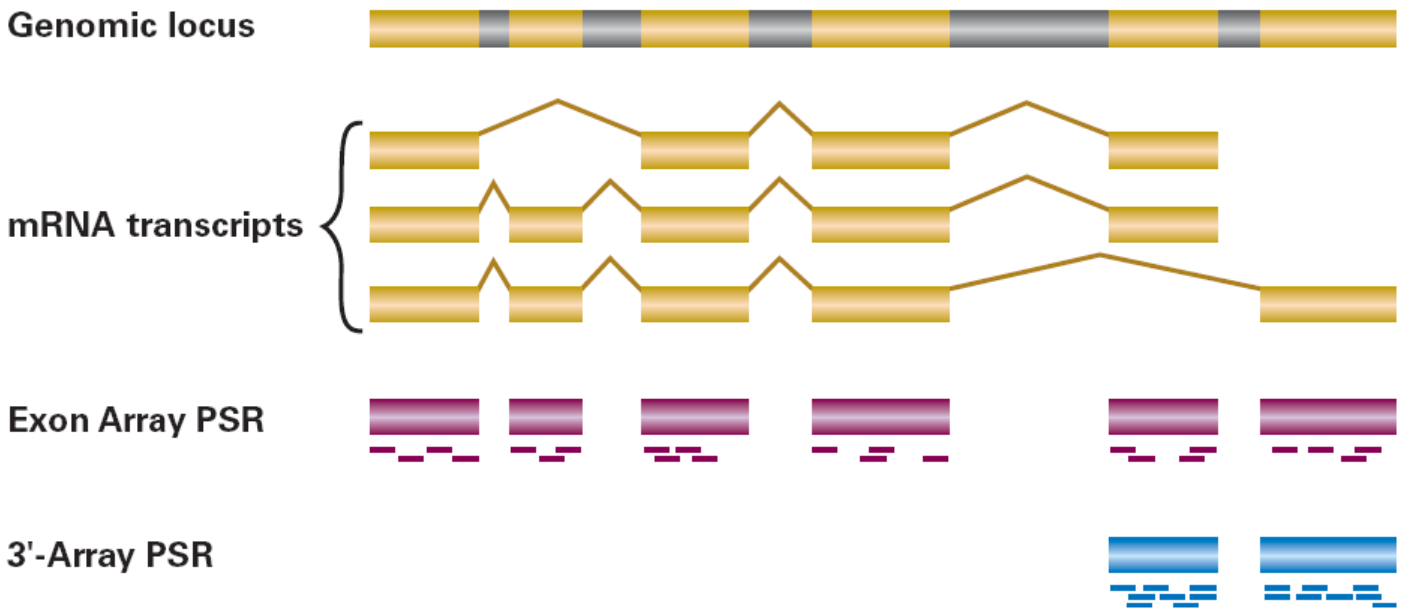
EXZELLENZ RWTH AACHEN

Exon Array Analysis

Array System:

Affymetrix GeneChip® Human Exon 1.0 ST Array
1.4 Millionen Probesets (> 1 Million Exoncluster)
Ensembl Transkripte + ESTs

(For comparison: Human Genome U133 Plus 2.0 Array > 54 000 Probesets)



www.affymetrix.com

Transcript Cluster ID	genesymbol	pvalue	FCAbsolute
3232979	AKR1C2|AKR1C1|LOC648517	6,98E-06	34,28
3274758	AKR1C2|AKR1C1|LOC648517|LOC648894	3,77E-06	22,34
2731332	IL8	7,69E-05	20,83
3233049	AKR1C3|AKR1C1|AKR1C2|LOC648517	1,03E-05	17,37
2962820	ME1|LOC731490	2,10E-05	12,46
3746845	TRIM16|CDRT1|TRIM16L	2,52E-05	11,47
2323847	PLA2G5	5,95E-05	11,36
2591421	TFPI	9,97E-04	10,99
2688955	CD200R1|CD200R2	9,90E-05	9,70
2362230	CD1E	1,77E-06	8,61
3719020	CCL4L1|LOC730424|LOC728835	1,04E-03	8,53
4040063	CCL4|CCL4L1|CCL4L2|LOC730424|LOC728835|LOC441792	8,70E-04	7,80
3718977	CCL4|CCL4L1|CCL4L2|LOC730424|LOC728835|LOC441792	6,08E-04	7,78
2880292	DPYSL3	1,09E-04	7,77
3233119	AKR1C4|AKR1C1|LOC648517	1,75E-04	7,76
2786322	SLC7A11	1,20E-05	7,63
3692701	CES1|LOC652708	1,39E-03	7,48
3791254	TNFRSF11A	1,03E-04	7,34
3266279	SLC18A2	6,70E-04	6,92
3718930	CCL4L2|CCL4L1	7,72E-04	6,75
2423625	GCLM	1,84E-05	6,45
3507282	FLT1	1,51E-03	6,21
2688933	CD200R2	2,76E-05	6,13
2343473	IFI44L	6,97E-04	6,11
2477073	CRIM1	1,08E-03	6,07
3057955	FGL2	1,74E-03	5,99
3540068	AKAP5	1,34E-03	5,87
3401704	CCND2	1,95E-04	5,72
3696666	NQO1	2,06E-04	5,69
3409605	MLSTD1	1,90E-03	5,56
3220673	LTB4DH|SLC11A1	7,62E-06	5,50
2362201	CD1C	5,57E-05	5,38
3742212	ALOX15	3,40E-03	5,34
4040932	CCL3L3|CCL3L1|LOC728830|LOC730422	9,73E-03	5,31
3718191	CCL8	3,10E-04	5,26
3279108	NMT2|LOC728322|LOC730874	4,70E-07	5,25

Exon Array Analysis

- Gene expression of human moDC after 30h incubation with TNBS (sensitizer) and CAld and CAlc (pooled data of 6 experiments: 3 TNBS vs. SDS, 3 CAld vs CAlc)
- **IL-8** is one marker gene
- >5-fold upregulation of 36 genes
- New marker genes?

TRIM 16 (EBBP)

Cell Death and Differentiation (2006) 13, 1938–1949

www.nature.com/cdd

The estrogen-responsive B box protein: a novel enhancer of interleukin-1β secretion

C Munding[1,3,4], M Keller[1,4], G Niklaus[1], S Papin[2], J Tschopp[2], S Werner[1] and H-D Beer*[,1]

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[2] Institute of Biochemistry, BIL Biomedical Research Center, University of Lausanne, CH-1066 Epalinges, Switzerland
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[4] These authors have equally contributed to this work
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Received 24.8.05; revised 14.2.06; accepted 15.2.06; published online 31.3.06
Edited by R De Maria

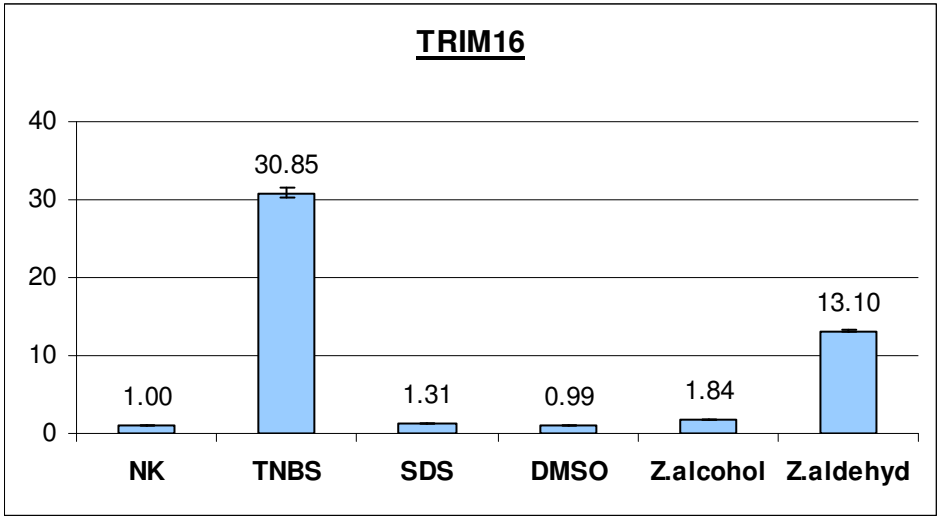
Abstract

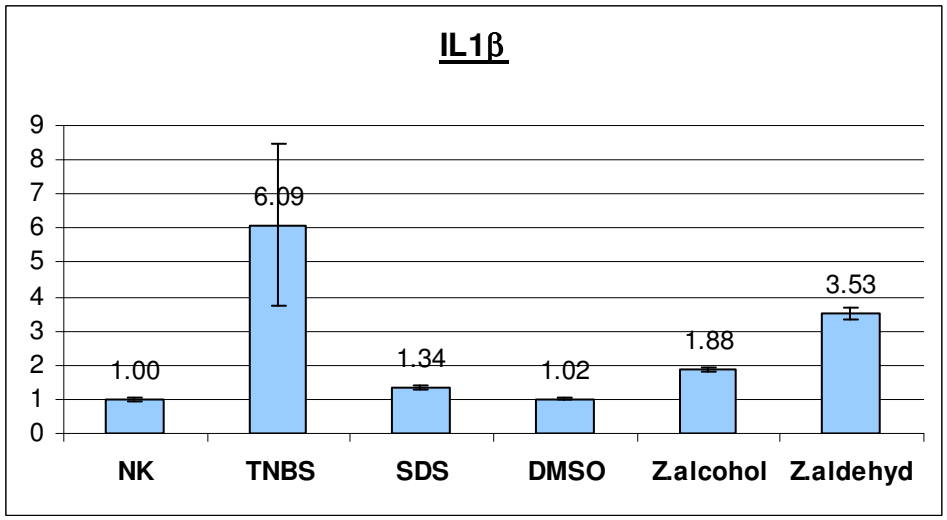
The estrogen-responsive B box protein (EBBP) and Pyrin belong to a family of structurally related proteins. While mutations in the *pyrin* gene cause an autoinflammatory disease, the biological function of EBBP is unknown. In this study, we identified the proinflammatory cytokine interleukin-1β (IL-1β) as an EBBP-binding partner. Furthermore, caspase-1 and NACHT, LRR and Pyrin domain containing protein (NALP) 1, two components of the recently identified inflammasome, a platform for the activation of caspase-1, also interact with EBBP. These proteins bind to the RFP domain of EBBP, suggesting that this domain of so far unknown function is an important protein-binding domain. EBBP was secreted in a caspase-1-dependent manner from cultured cells, and its secretion was enhanced by IL-1β. Vice versa, endogenous and overerexpressed EBBP increased IL-1β secretion. These results provide evidence for a role of EBBP in innate immunity by enhancing the alternative secretion pathway of IL-1β.

Cell Death and Differentiation (2006) **13**, 1938–1949.
doi:10.1038/sj.cdd.4401896; published online 31 March 2006

activates lymphocytes, recruits leukocytes and is involved in the generation of fever. Based on these activities, it functions as a central mediator in various acute and chronic inflammatory diseases, thus representing a potential target for therapeutic intervention (reviewed by Dinarello[1,2]).

Initial synthesis of this cytokine occurs as an inactive precursor (proIL-1β), which lacks a signal sequence. Nevertheless, active IL-1β and proIL-1β are released by a poorly characterized mechanism, particularly from activated macrophages (reviewed by Dinarello[1]). Secretion is independent of the Golgi apparatus and can be stimulated by exogenous ATP via the P2X7 receptor.[3–5] Processing and activation of proIL-1β is strictly dependent on the protease caspase-1, which cleaves proIL-1β after the aspartic acid residue at position 116, generating active IL-1β.[6,7] Consequently, macrophages from caspase-1 knockout mice cannot produce mature IL-1β.[8] Caspase-1 itself is expressed as an inactive precursor. Recently, it was shown that activation of procaspase-1 and subsequent processing of proIL-1β in a cell-free system from macrophages is dependent on a protein complex called the inflammasome.[9] It consists of procaspases-1 and -5, Nacht, LRR and Pyrin domain containing protein (NALP)1[10,11] and apoptosis-associated speck-like protein containing a card (Asc).[12] The assembly of this complex relies only on homotypic interactions of the death domain fold family member caspase recruitment domain (CARD) and the recently identified pyrin domain.[11,13,14] The NALP1 protein contains an amino-terminal pyrin domain and a carboxy-terminal CARD (Figure 2a). The latter allows binding to the CARD of procaspase-5, whereas the pyrin domain binds via the bipartite adaptor protein Asc, which consists only of a pyrin domain and a CARD, to the CARD of procaspase-1 (Figure 2a). Thereby, procaspase-1 and -5 come in close proximity, allowing their activation.[9] All components of the inflammasome are constitutively expressed in macrophages, but the signals, which stimulate the assembly and activation of the inflammasome, are as yet unknown.[9] In addition, it is unclear if there is a link between procaspase-1 activation and





AKR1

0026-895X/06/6905-1662–1672$20.00
MOLECULAR PHARMACOLOGY
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Mol Pharmacol 69:1662–1672, 2006

Vol. 69, No. 5
19794/3110693
Printed in U.S.A.

Induction of *AKR1C2* by Phase II Inducers: Identification of a Distal Consensus Antioxidant Response Element Regulated by NRF2

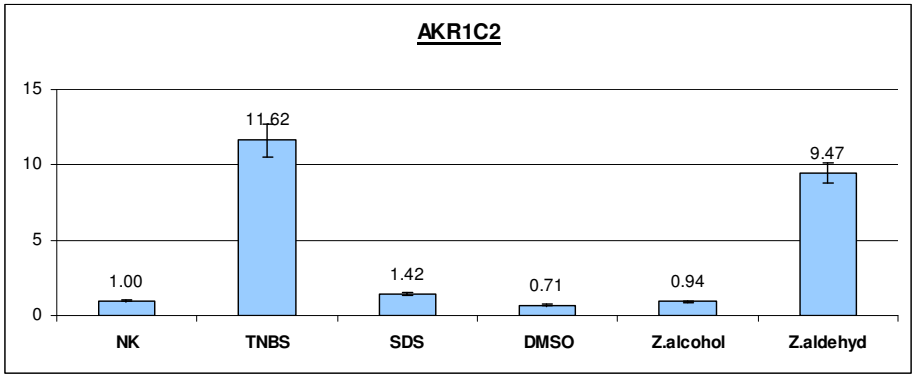
Huan Lou, Shouying Du, Qing Ji, and Andrew Stolz

Division of Gastrointestinal and Liver Diseases, Department of Medicine, Keck School of Medicine of the University of Southern California, Los Angeles, California

Received October 11, 2005; accepted February 14, 2006

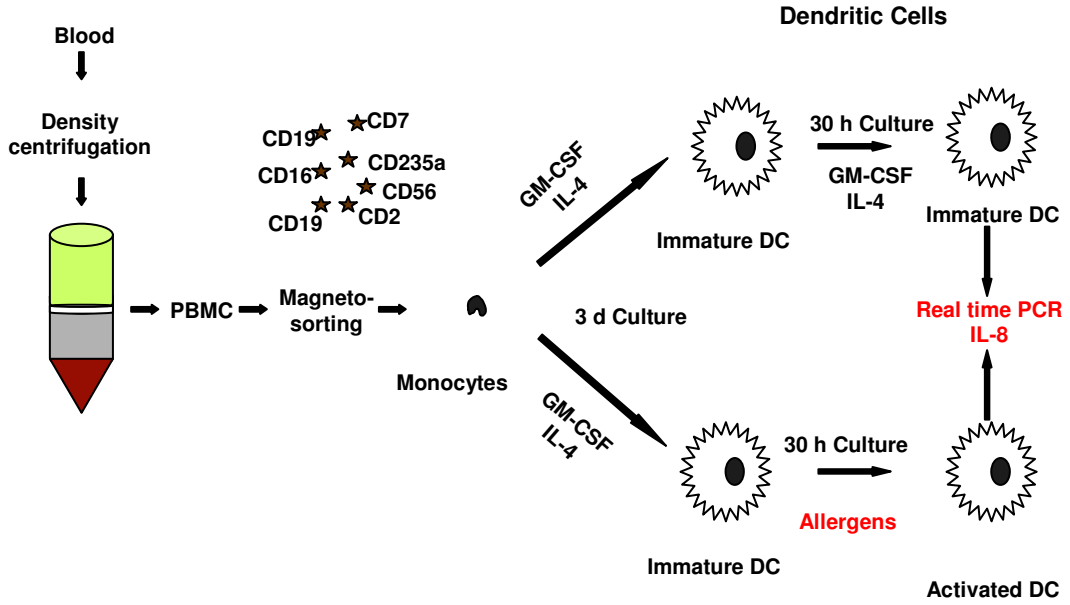
ABSTRACT

AKR1C2, also referred to as the human bile acid binder and 3α-hydroxysteroid dehydrogenase type III, is a multifunctional oxidoreductase able to stereoselectively reduce steroids as well as oxidize or reduce polyaromatic hydrocarbons. Previously, this same protein was also identified by its robust induction by phase II inducers in HT29 cells. In HepG2 cells, both *AKR1C2* and *AKR1C1* (97% sequence homology) were induced by phase II inducers but not the highly related *AKR1C3* and *AKR1C4* family members (84% sequence homology). We now report the initial characterization of the proximal promoter of *AKR1C2* in HepG2 cell line and the identification of a potent enhancer-like element responsive to phase II inducers located approximately 5.5 kilobases upstream from the transcription start site. DNA sequence analysis of this enhancer element revealed that it contained a consensus antioxidant response element (ARE), which was confirmed by mutation analysis. Treatment with phase II inducers leads to increased accumulation of nuclear factor-erythroid 2 p45-related factor (NRF) 2 in the nucleus, which was associated with increased binding to this ARE as determined by electrophoretic mobility shift assay. Transient transfection with *Nrf2* increased the transcriptional activity of the ARE of *AKR1C2* comparable with that observed with phase II inducers. Chromatin immunoprecipitation (ChIP) analysis also confirmed increased NRF2 binding to the ARE after induction by a phase II inducer. The *AKR1C1* promoter also harbored this same ARE element in a highly homologous region, which was also bound by NRF2 in a ChiP analysis. No induction of the ARE of *AKR1C2* was detected in Nrf2$^{-/-}$ fibroblasts. The regulation of *AKR1C2* by this distal ARE suggests that AKR1C2 detoxifies products of reactive oxidant injury, which has important implications for both hormone and xenobiotic metabolism.

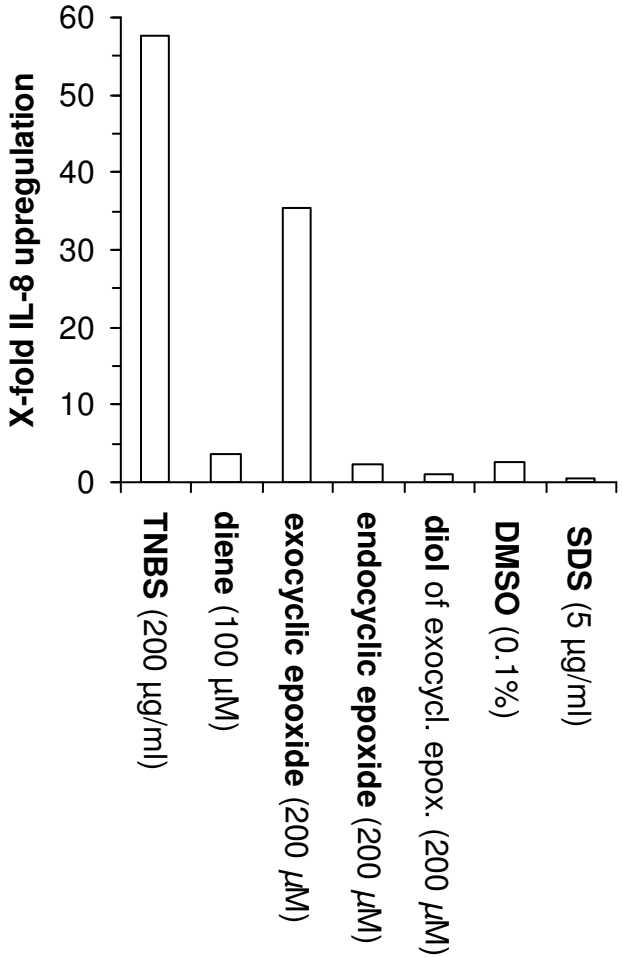


Sensitizing capacity in dendritic cell (DC) assay

- In vitro sensitizing capacity of the diene, the epoxides and the diol in a **dendritic cell (DC) assay** (Aeby P et al., *J Invest Dermatol*, 2004)

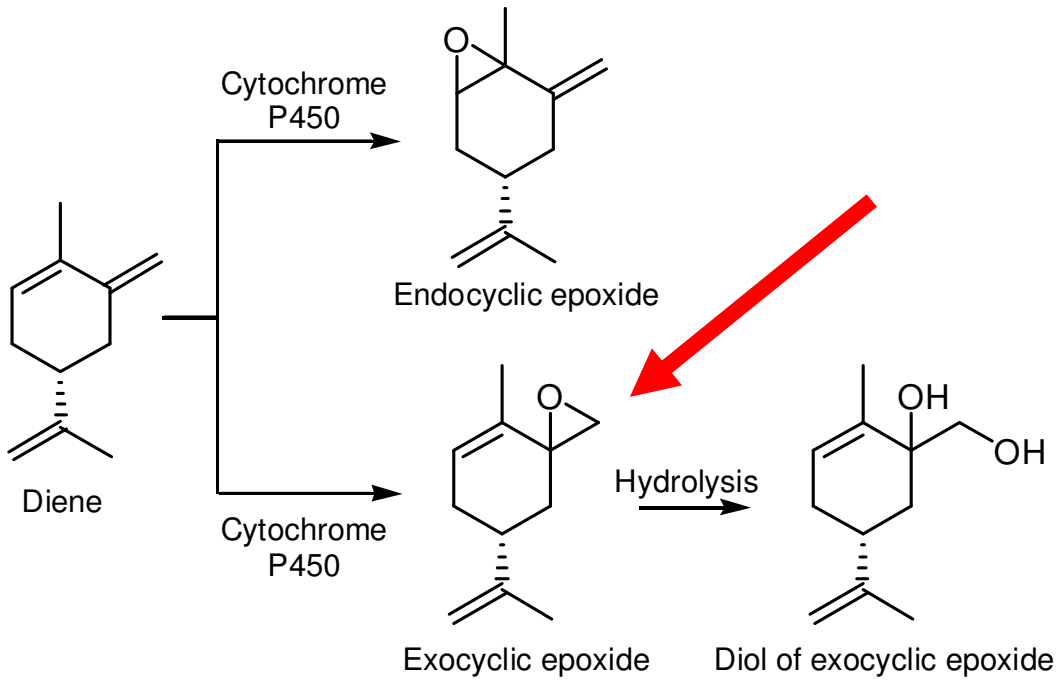


IL-8 mRNA expression in immature **DC** after **incubation** for 30h



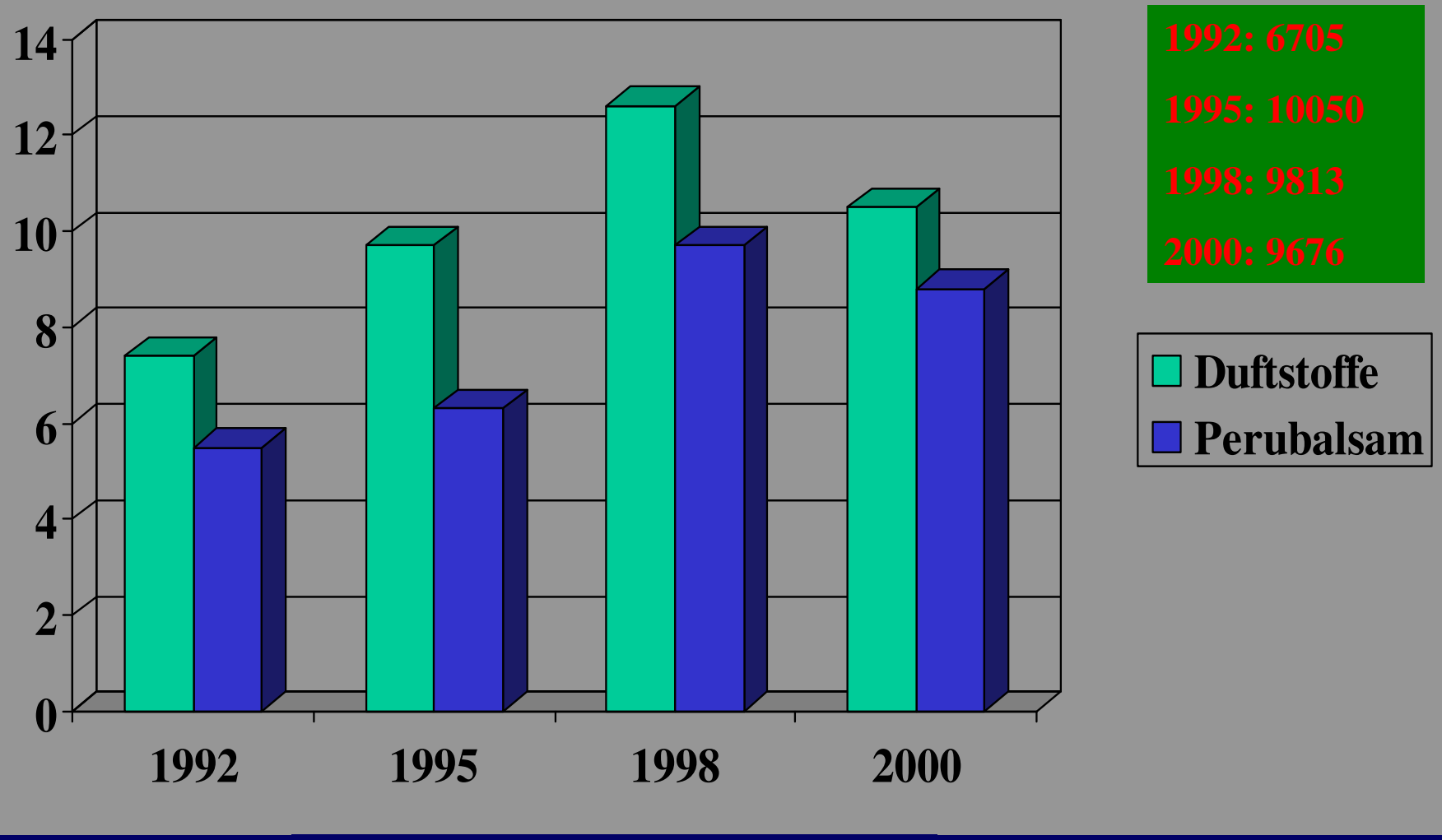
Model prohapten for metabolism studies

- Cytochrome P450-mediated metabolism of a **model prohapten** the conjugated diene ((5R)-5-isopropenyl-2-methyl-1-methylene-2-cyclohexene) to an **endocyclic** and **exocyclic epoxide** followed by hydrolysis of the exocyclic epoxide to a **diol**



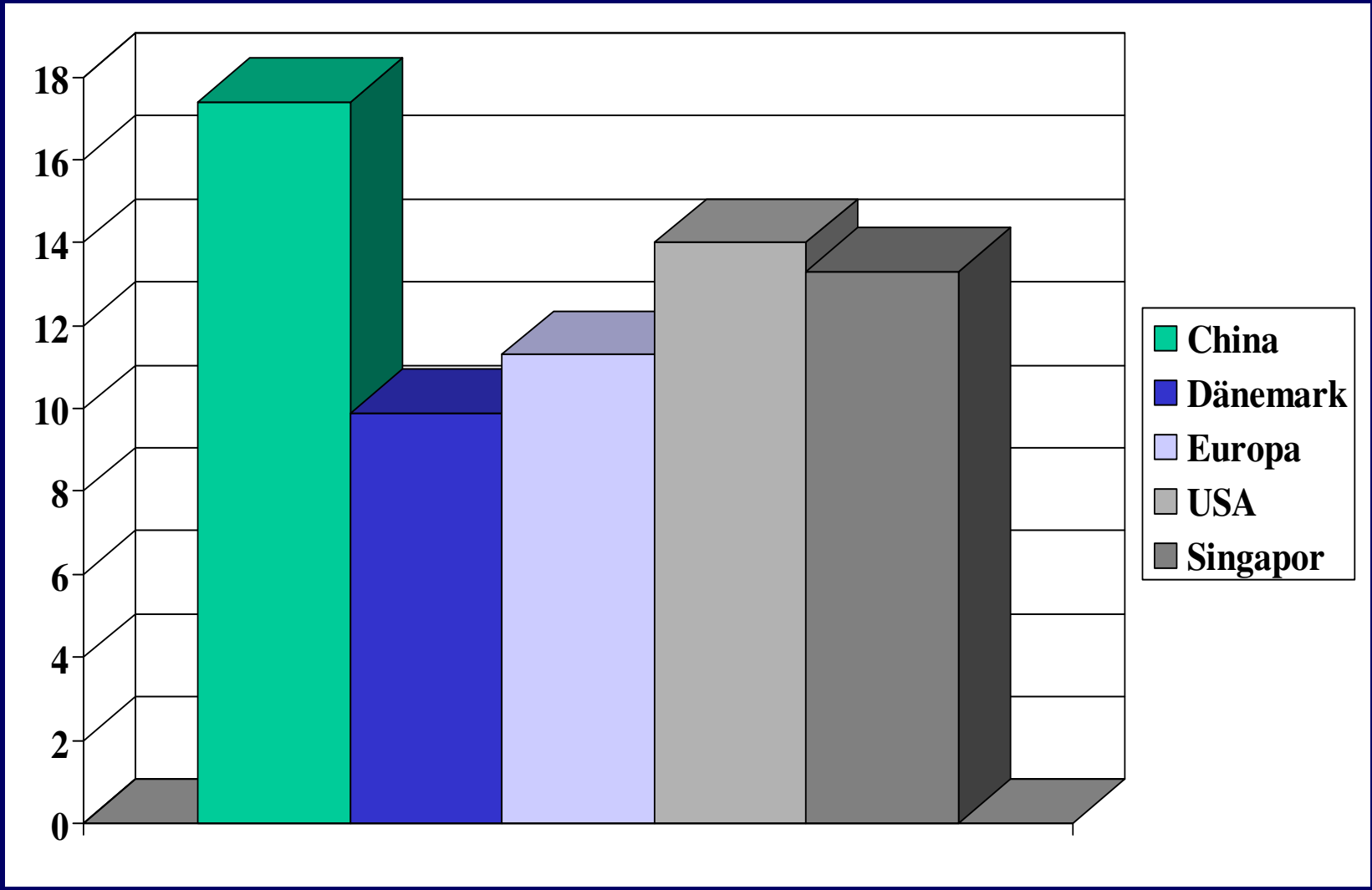
Chem. Res. Toxicol 18:308-16,2005
J Invest Dermatol, 127: 1145–1153, 2007

Duftstoff-Sensibilisierung 1992 - 2000



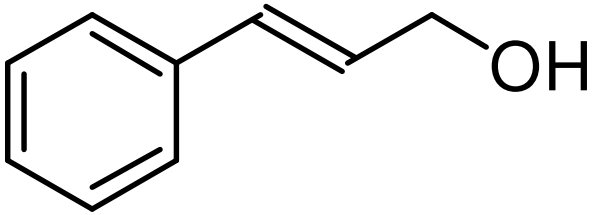
Schnuch et al., 2002 [IDVK]

Duftstoff-Sensibilisierung weltweit



Johansen, 2002

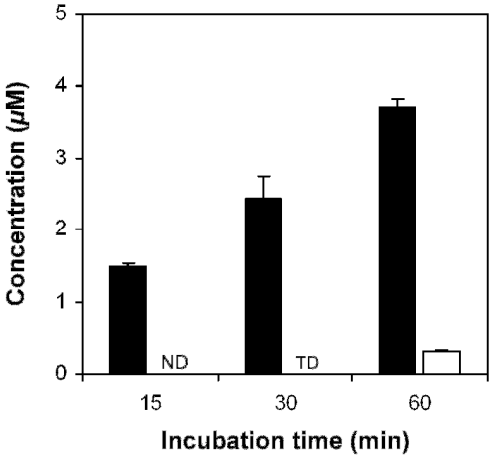
Activation of cinnamic alcohol is mediated by CYPs expressed in skin cells



Cinnamic alcohol
Prohapten

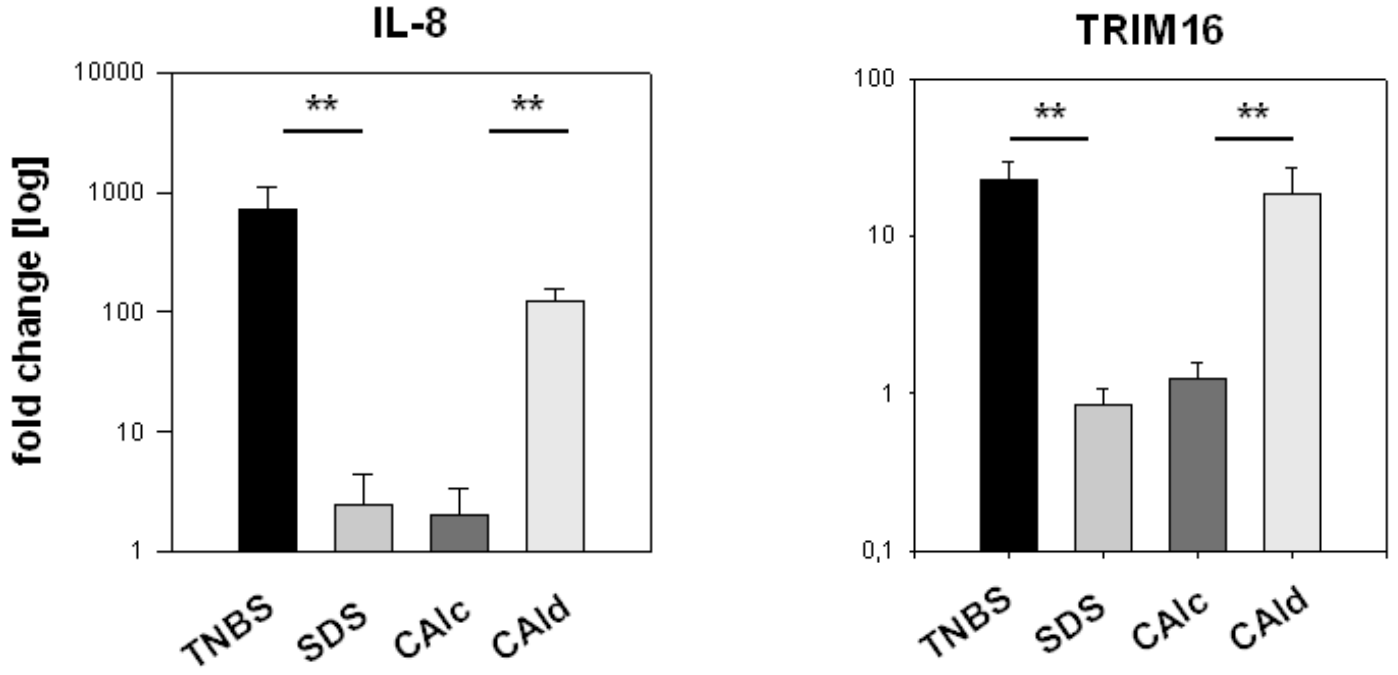
Incubation with skin specific rhCYP cocktail

Detection of cinnamic aldehyde(■) and cinnamic acid (□) by LC-MS



J Invest Dermatol, 127: 1145–1153, 2007
Skin Pharmacol Physiol, 23(4):213-224, 2010

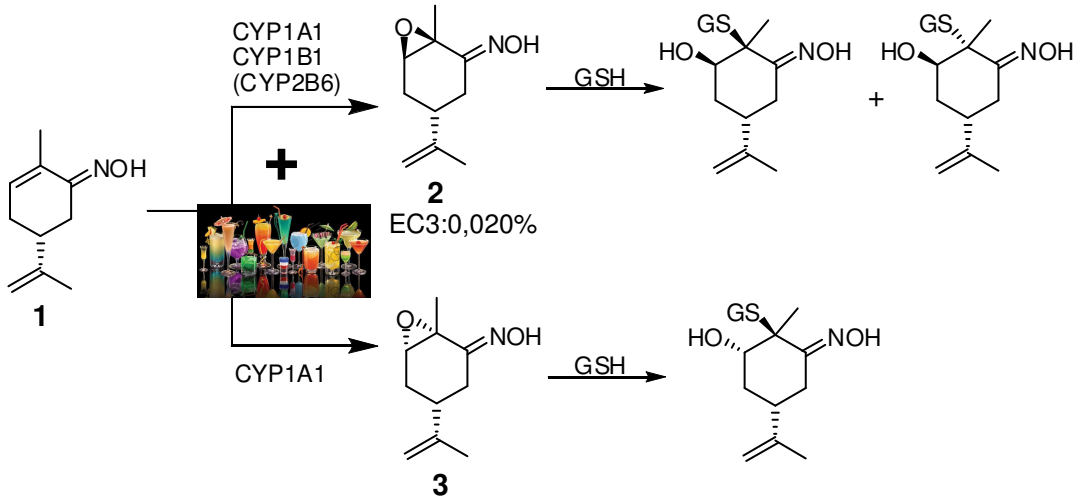
Influence of prohaptens and haptens on the activation of immature dendritic cells

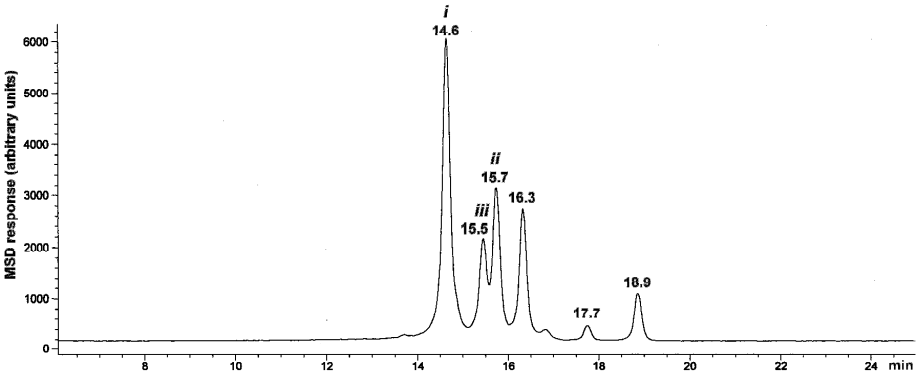


Skin Pharmacol Physiol, 23(4):213-224, 2010

Activation of R-carvoxime is mediated by CYPs expressed in skin cells

CYP mediated epoxidation of the **prohapten** α,β-unsaturated oxime **R-carvoxime** and subsequent conjugation with glutathione (GSH)





Chem Res Toxicol, 20:927-936, 2007
Chem Res Toxicol, 22(2):399-405, 2009

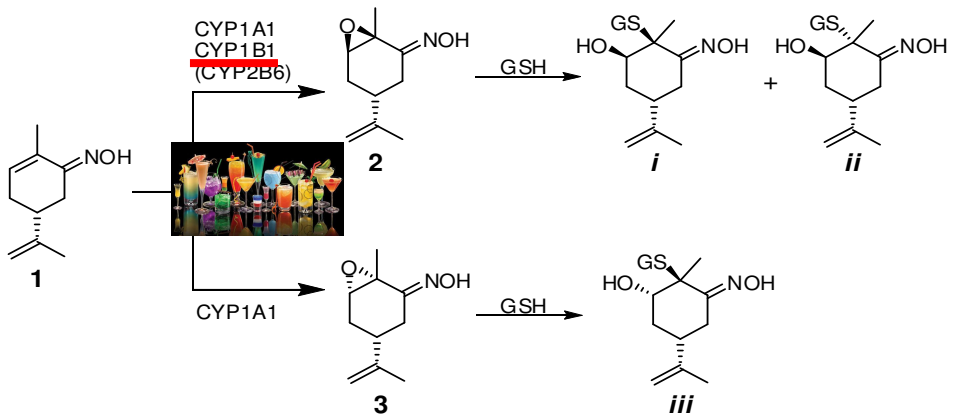
LC-MS chromatograms of glutathione conjugates formed from R-carvoxime **using the skin-like cytochrome P450 cocktail**

Metabolic activation of Prophapten R-Cavoxime

Cutaneous Metabolic Activation of Carvoxime, a Self-Activating, Skin-Sensitizing Prohapten

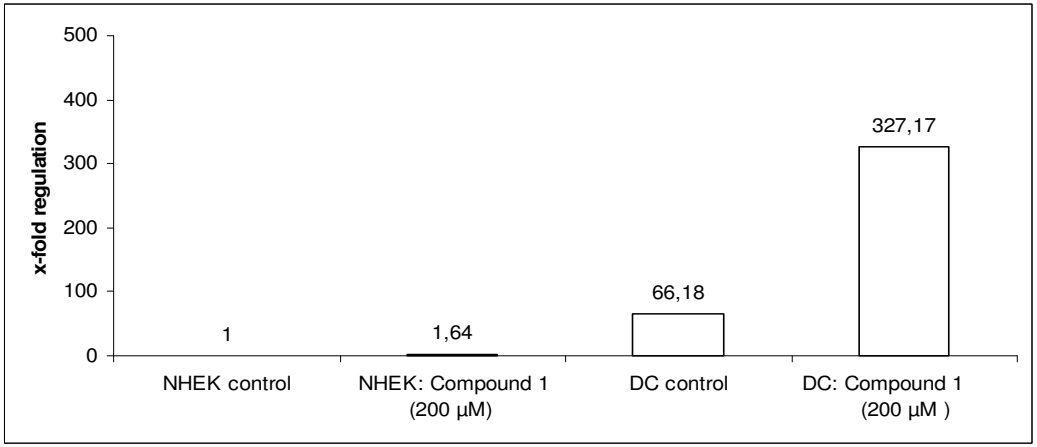
Hagen Ott[a], Moa Andresen Bergström[b], Ruth Heise[a], Claudia Skazik[a], Gabriele Zwadlo-Klarwasser[c], Hans F. Merk[a], Jens M. Baron[a] and Ann-Therese Karlberg[b]

Chem Res Toxicol, 22(2): 399-405, 2009



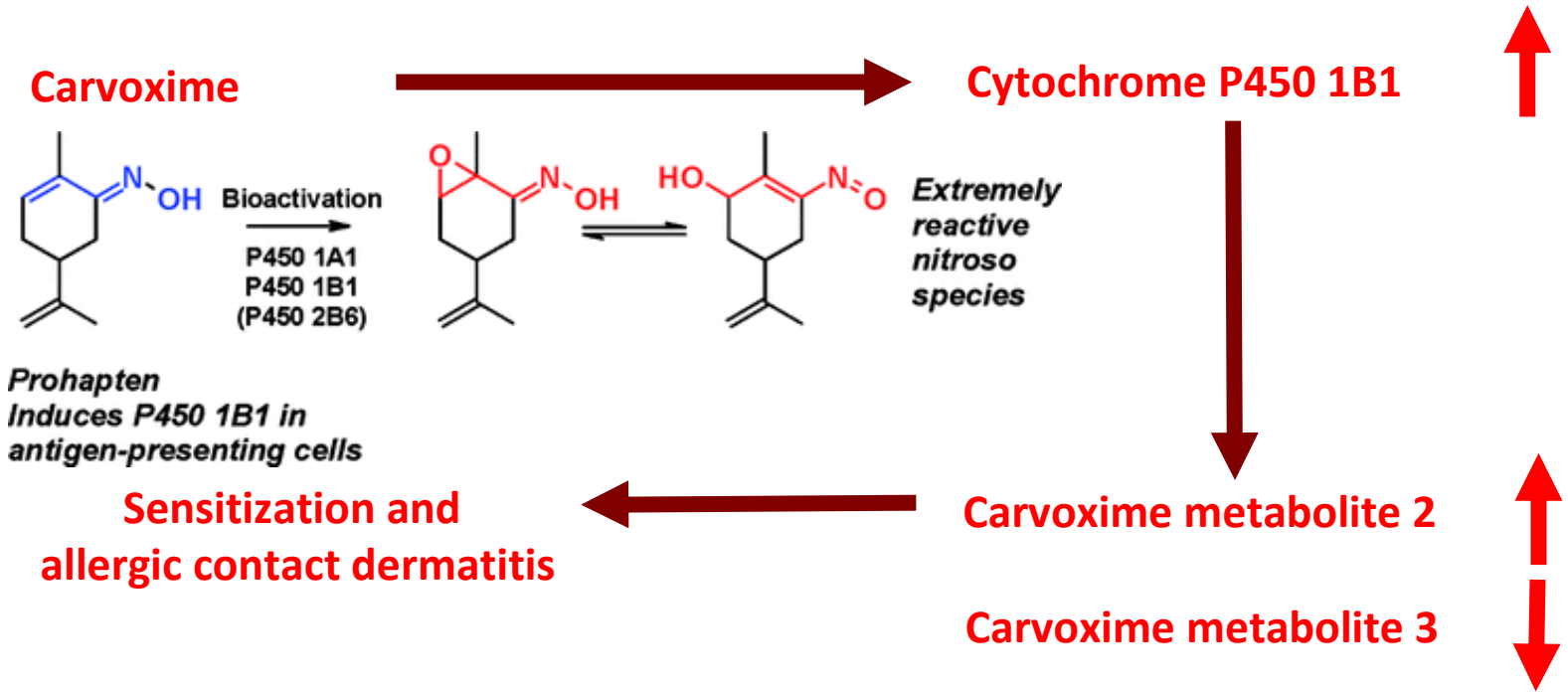
CYP mediated epoxidation of the **prohapten** α,β-unsaturated oxime **R-carvoxime** and subsequent conjugation with glutathione (GSH)

Prohapten (R-Carvoxime) can **stimulate** its CYP-mediated **metabolism**

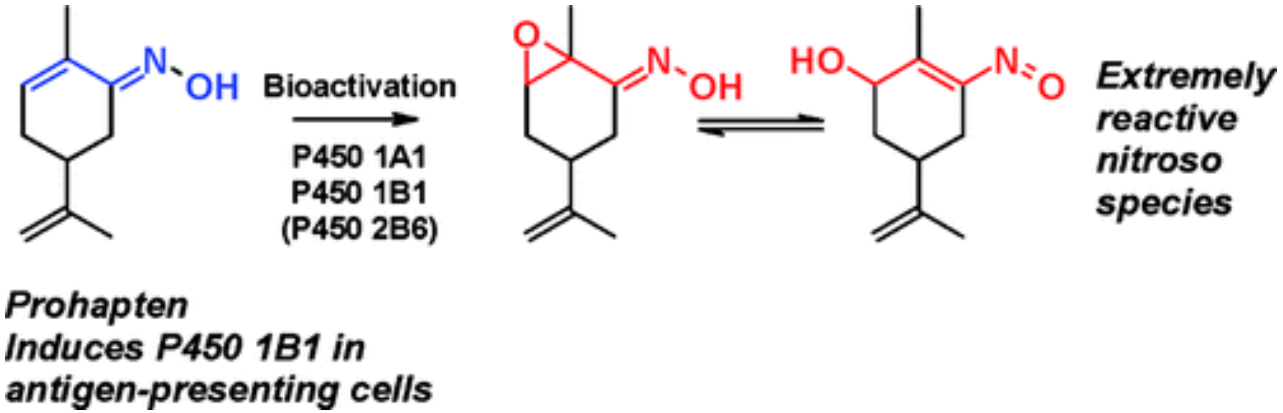


EXZELLENZ RWTH AACHEN

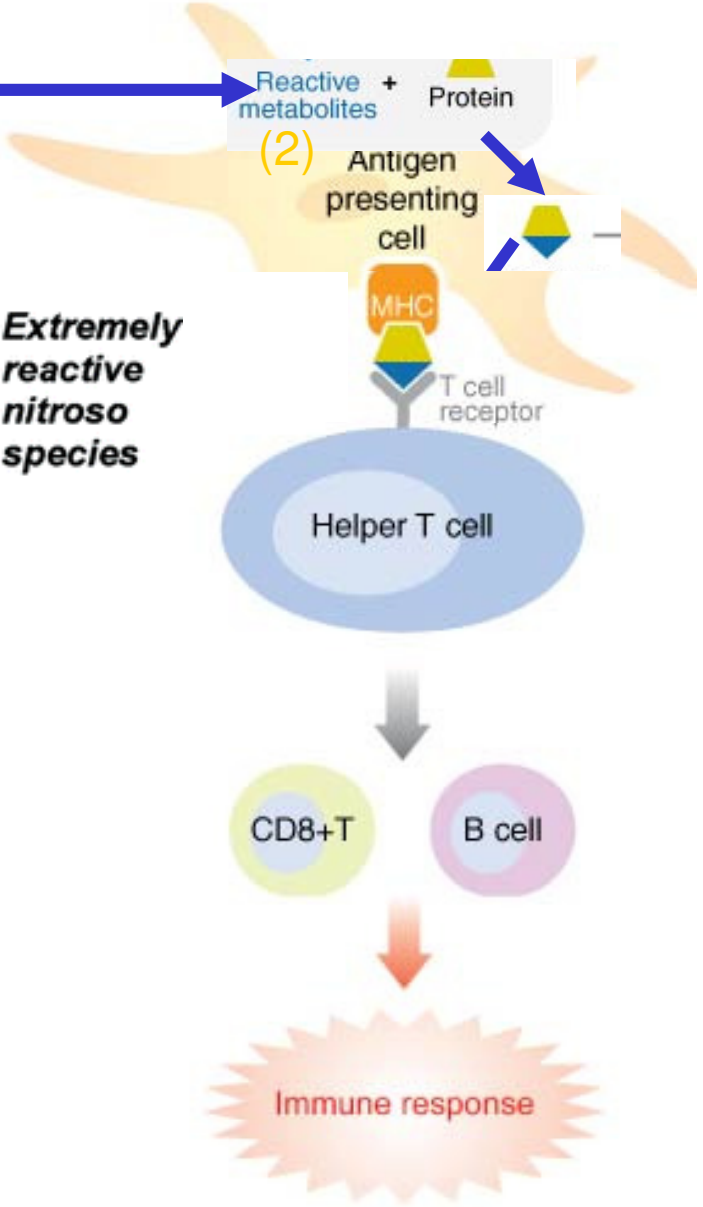
Parent compound may induce its own metabolism to the nominative antigen in antigen presenting dendritic cells



Carvoxime (1)



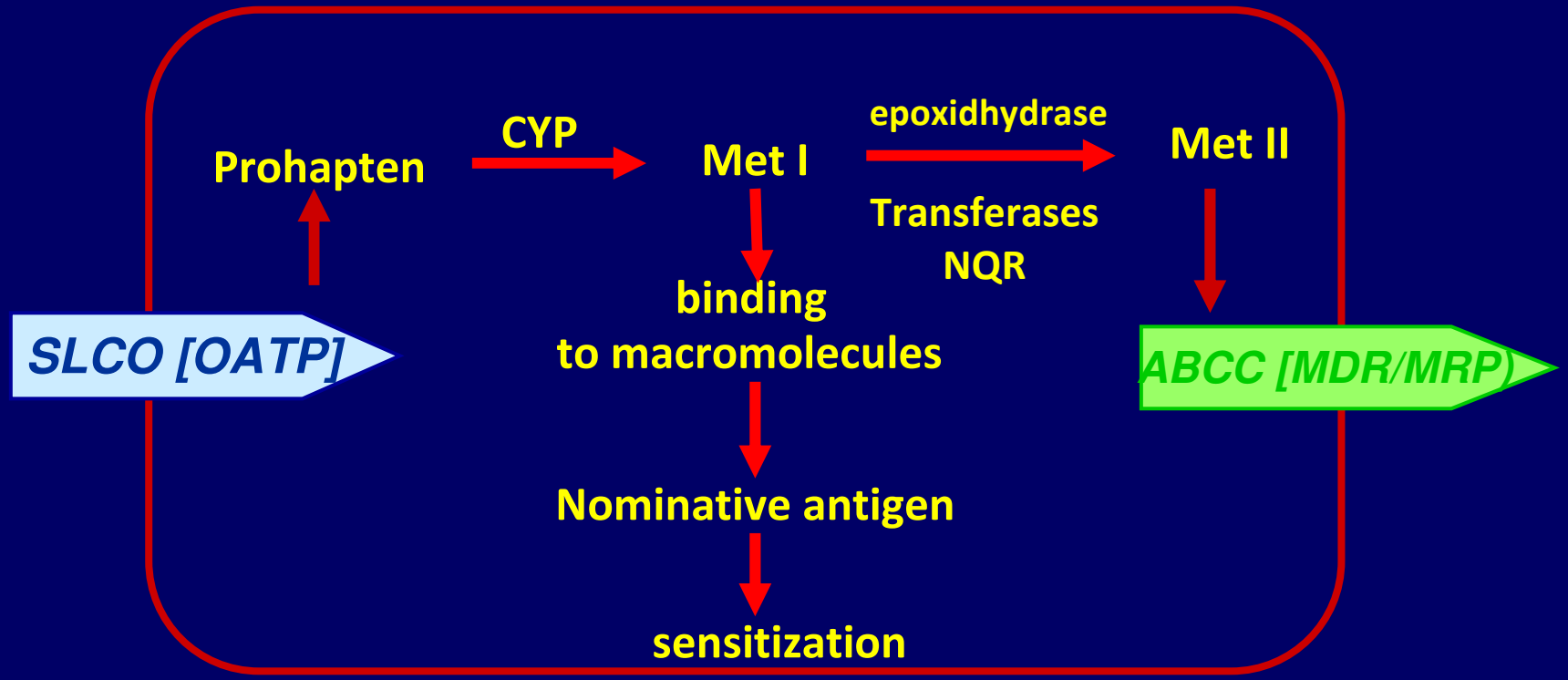
Prohapten
Induces P450 1B1 in antigen-presenting cells



Adapted from Uetrecht et al., 2007 and H Ott et al, 2009

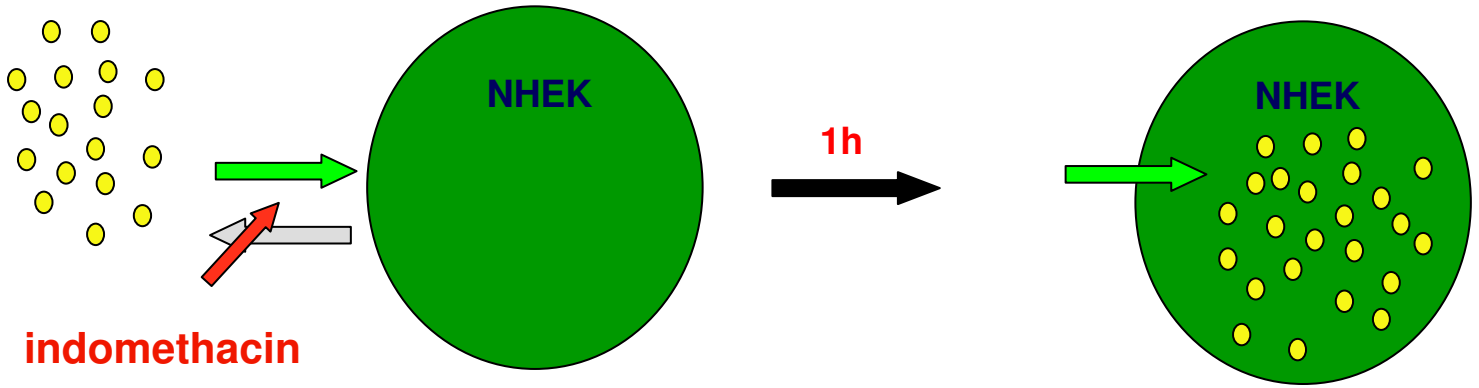
Metabolism/ Toxicity of xenobiotica

Phase I: CYP – **Phase II:** EH/ Transferases –
Phase III: Transporter proteins



ABCC: ATP binding cassette C transporters (MRP/ MDR)
SLCO: solute carrier organic anion transporter (OATP)

Transport assay



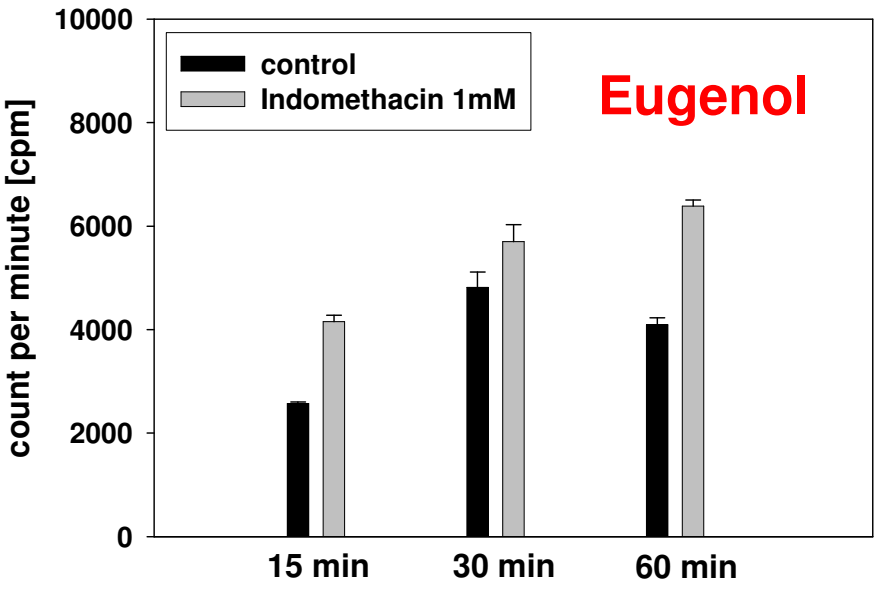
indomethacin

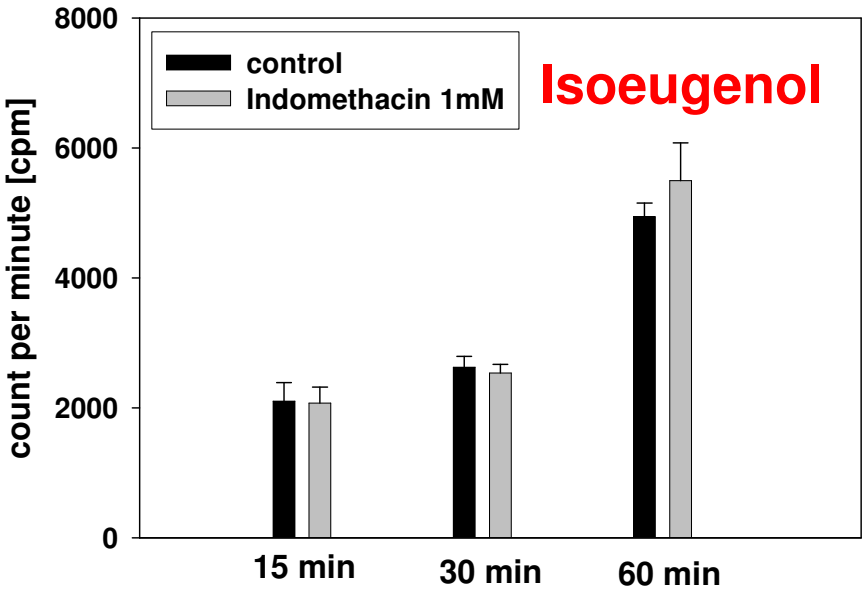
- specific MRP inhibitor
- concentration of 1 mM and 200 µM

scintillation buffer

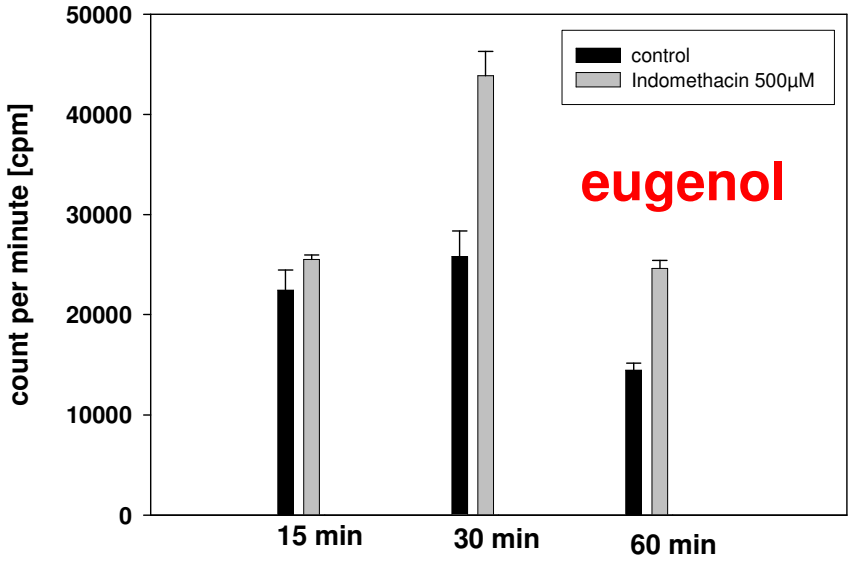
Measurement of cell-associated radioactivity

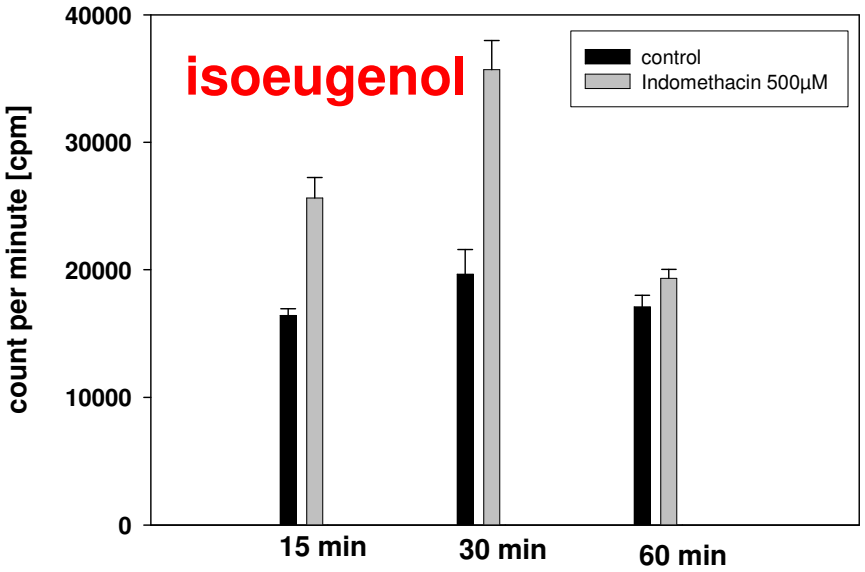
Inhibitory effect of Indomethacin on MRP-mediated efflux in NHEKs





Inhibitory effect of Indomethacin on MRP-mediated efflux in moDCs





Human moDC in vitro assay

Characterization of the Sensitizing Potential of Chemicals by *In Vitro* Analysis of Dendritic Cell Activation and Skin Penetration

Pierre Aeby,* Christoph Wyss,* Heinz Beck,* Peter Griem,§ Heike Scheffler,† and Carsten Goebel†

J Invest Dermatol 122:1154–1164, 2004

High-Resolution Transcriptional Profiling of Chemical-Stimulated Dendritic Cells Identifies Immunogenic Contact Allergens, but Not Prohaptens

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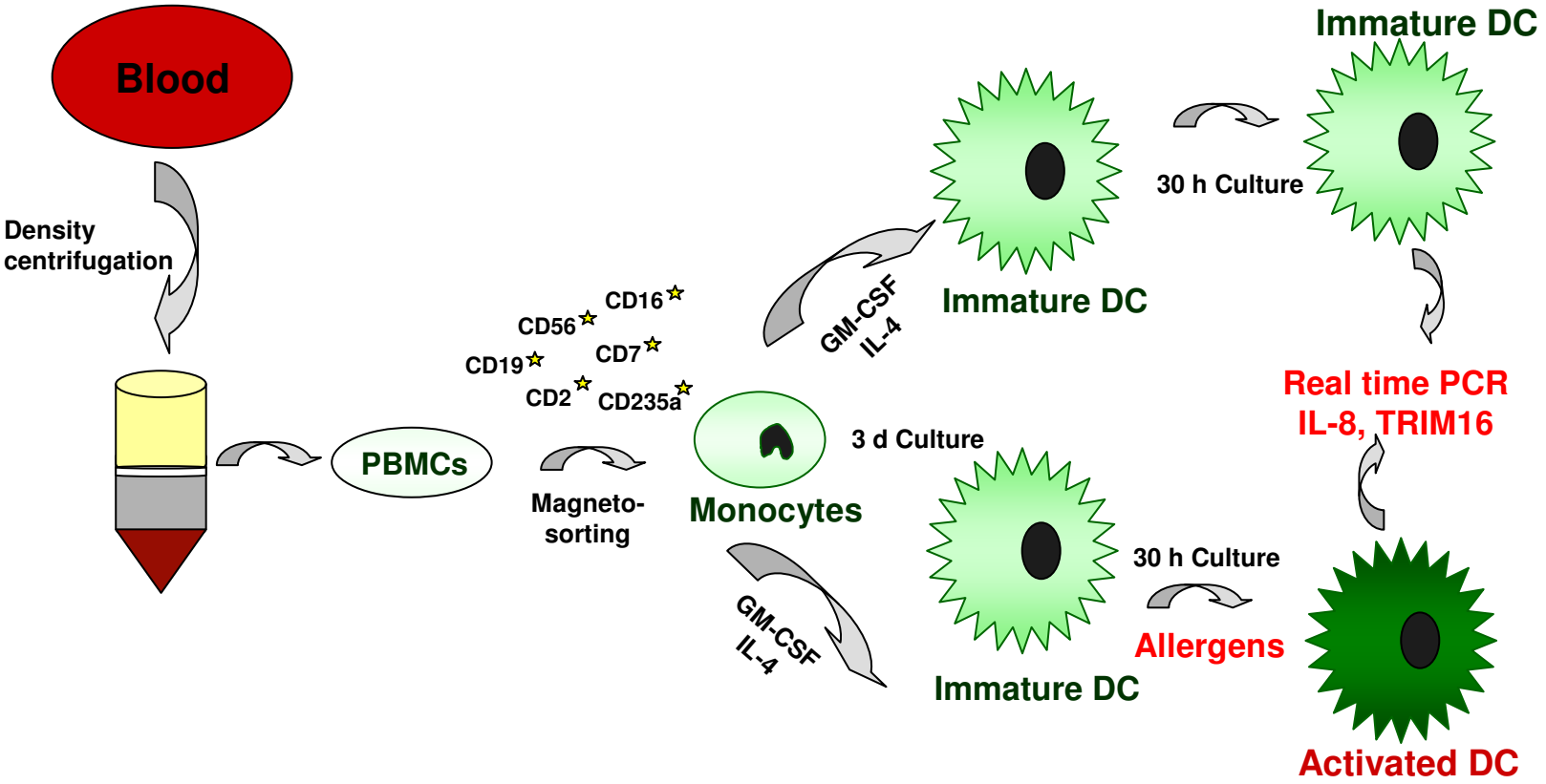
Skin Pharmacol Physiol 2010;23:213–224



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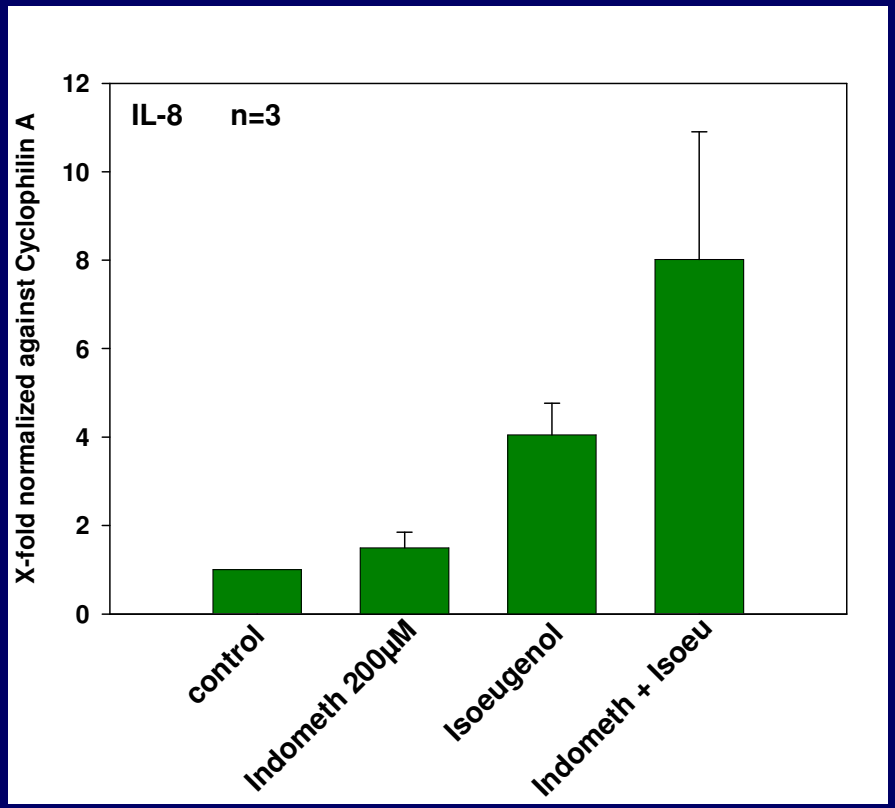
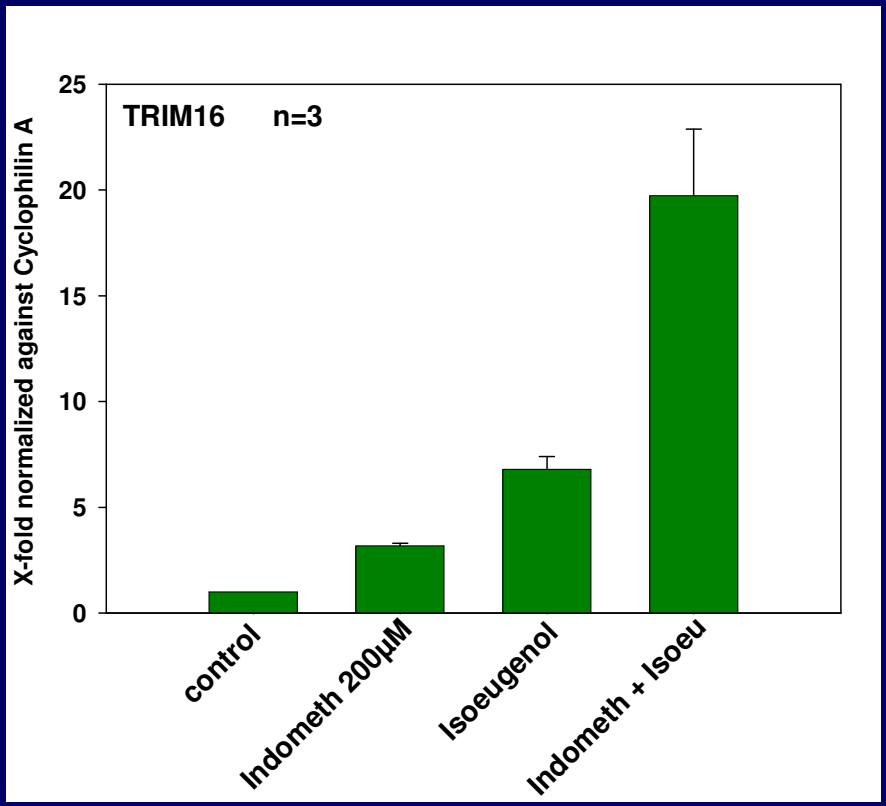
IL-8 and TRIM16 mRNA upregulation after treatment with Isoeugenol + Indomethacin





Acknowledgment

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This work was supported in part by a grant from the European Commission as part of the integrated project `Novel Testing Strategies for In Vitro Assessment of Allergens (Sens-it-iv) LSHB-CT-2005-018681 and a grant from Deutsche Forschungs-gemeinschaft (SFB 542, TP C11)

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