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Review

Progress on the development of human *in vitro* dendritic cell based assays for assessment of the sensitizing potential of a compoundG. Galvão dos Santos^a, J. Reinders^a, K. Ouwehand^a, T. Rustemeyer^a, R.J. Scheper^b, S. Gibbs^{a,*}^a Department of Dermatology, VU University Medical Centre, Amsterdam, The Netherlands^b Department of Pathology, VU University Medical Centre, Amsterdam, The Netherlands

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ABSTRACT

Allergic contact dermatitis is the result of an adaptive immune response of the skin to direct exposure to an allergen. Since many chemicals are also allergens, European regulations require strict screening of all ingredients in consumer products. Until recently, identifying a potential allergen has completely relied on animal testing (e.g.: Local Lymph Node Assay). In addition to the ethical problems, both the 7th Amendment to the Cosmetics Directive and REACH have stimulated the development of alternative tests for the assessment of potential sensitizers. This review is aimed at summarising the progress on cell based assays, in particular dendritic cell based assays, being developed as animal alternatives. Primary cells (CD34⁺ derived dendritic cells, monocyte derived dendritic cells) as well as dendritic cell-like cell lines (THP-1, U-937, MUTZ-3, KG-1, HL-60, and K562) are extensively described along with biomarkers such as cell surface markers, cytokines, chemokines and kinases. From this review, it can be concluded that no single cell based assay nor single marker is yet able to distinguish all sensitizers from non-sensitizers in a test panel of chemicals, nor is it possible to rank the sensitizing potential of the test chemicals. This suggests that sensitivity and specificity may be increased by a tiered assay approach. Only a limited number of genomic and proteomic studies have been completed until now. Such studies have the potential to identify novel biomarkers for inclusion in future assay development. Although progress is promising, this review suggests that it may be difficult to meet the up and coming European regulatory deadlines.

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Q152 Introduction

53 Allergic contact dermatitis (ACD) is an adaptive inflammatory
 54 response of the skin after exposure to an allergen. It is a T cell-
 55 mediated disease and recognised as a delayed-type hypersensitivity
 56 immune response (type IV) (Smith and Hotchkiss, 2001; Moed et al.,
 57 2004; Rustemeyer et al., 2004).

58 Chemical allergens are small molecules which are present in the
 59 environment and in many household products and which can form a
 60 sensitizing complex once bound to a skin protein (Weltzien et al.,
 61 1996). After contact with the allergen, sensitization can occur.
 62 Repeated contact with the same allergen after sensitization results
 63 in elicitation and symptoms of ACD (Smith and Hotchkiss, 2001). It is
 64 often difficult clinically and histologically to distinguish ACD from
 65 irritant contact dermatitis (ICD) since the inflammatory mechanisms
 66 are quite similar (Smith and Hotchkiss, 2001; Levin and Maibach,
 67 2002; Smith et al., 2002; Bonneville et al., 2007). However since ACD is
 68 an adaptive immune response and ICD is an innate immune response
 69 it is thought that in the future *in vitro* assays will be able to distinguish
 70 a sensitizer from a non-sensitizer. Currently, one of the most
 71 promising of these are dendritic cell (DC) based assays since DC play
 72 a pivotal role in adaptive immune responses. It is the progress in the
 73 development of these DC based assays which are the subject of this
 74 review. Another promising *in vitro* approach is based on the ability of a
 75 chemical to react with proteins containing cysteine and lysine thus
 76 forming stable (covalent) bonds. This non-cell based assay classifies
 77 minimum reactivity as non-sensitizers and low, moderate and high
 78 reactivity as sensitizers and has been extensively described in a recent
 79 review (Gerberick et al., 2007 and 2008).

80 During allergen contact with the skin, immature cutaneous
 81 dendritic cells (iDCs), known as Langerhans cells (LCs), recognise
 82 and internalize the hapten-protein complex. LC then loose their
 83 potential to capture new antigens and gain the potential to present the
 84 hapten-protein complex to naïve T cells – this is commonly known
 85 as DC maturation. Simultaneously, under the influence of epidermal
 86 cytokines (e.g.: IL-1 α , IL-1 β , TNF- α , IL-18) and fibroblast- blood
 87 endothelial- and lymph endothelial-chemokines (CXCL12, CCL19,
 88 CCL21), the maturing LC migrate from the epidermis to the dermis
 89 and then to the draining lymph nodes where they can present the
 90 antigen to T-cells (Enk and Katz, 1992; Cumberbatch et al., 2001;
 91 Cumberbatch et al., 2003; Martín-Fontecha et al., 2003; Antonopoulos
 92 et al., 2008; Ouwehand et al., 2008). DC activation upon allergen
 93 exposure also leads to functional changes in DC, such as changes in
 94 cytokine (TNF- α IL-1 β) and chemokine secretion (De Smedt et al.,
 95 2002; Toebak et al., 2006a; Toebak et al., 2006b), and to changes in
 96 the expression of chemokine receptors (CCR7, CXCR4) (Rustemeyer
 97 et al., 2003; Staquet et al., 2004; Jugde et al., 2005). Many co-
 98 stimulatory molecules and intercellular adhesion molecules (e.g.:
 99 HLA-DR, HLA-ABC, CD40, CD80, CD83, CD86, ICAM-1/CD54), are up-
 100 regulated during DC maturation (Rambukkana et al., 1995; Ozawa
 101 et al., 1996; Aiba et al., 1997). Such biomarkers may have the potential
 102 in the future to distinguish a sensitizer from a non-sensitizer in an
 103 *in vitro* DC based assay.

104 DC based assays, using DC derived from fresh blood cell
 105 progenitors or DC-like cell lines are one of the most promising
 106 approaches to distinguish a sensitizer from a non-sensitizer. This is
 107 because once these cells are exposed to a sensitizer phenotypic
 108 alterations (e.g. increase in cytokine and chemokine secretion, and up-
 109 regulation of co-stimulatory molecules) that resemble DC maturation

processes occur in a similar manner to that observed *in vivo*. The focus
 of this review describes the need for the development of *in vitro*
 assays and in particular concentrates on the current status of DC based
 assays under development.

Current animal models and the need for animal alternatives

Identification of a potential sensitizer currently completely relies
 on animal testing. The mouse Local Lymph Node Assay (LLNA) is the
 most frequently used and accurate test with regard to relevance
 (predictive capacity) and reliability (reproducibility within and
 between laboratories) in distinguishing a sensitizer from a non-
 sensitizer (Kimber et al., 2002; Basketter and Cadby, 2004; Gerberick
 et al., 2004). This is closely followed by the Guinea pig maximization
 test (Basketter and Scholes, 1992).

Since the mid-1980s efforts to achieve a reduction in animal
 testing have slowly been gaining momentum in Europe. This was
 initiated by pressure applied from animal welfare groups, public
 opinion and ethical debates. In 1986, an EU directive on the protection
 of animals used for experimental purposes (Directive 86/609/EEC)
 (European Commission (1986) banned the use of an animal experi-
 ment when a scientifically approved alternative exists. The 7th
 Amendment to the Cosmetics Directive (Directive 76/768/EEC)
 foresees a complete ban on animal testing for cosmetic ingredients
 from 2009 for all the human health-related effects. Finally, incon-
 sistent with the above mentioned directives, the REACH legislation
 (Registration, Evaluation and Authorization of Chemicals) (European
 Regulation (2006) will soon be implemented, resulting in an increase
 in the number of animal experiments to be carried out for risk
 assessment of nearly 30,000 chemicals already marketed in the EU.
 All in all, this has resulted in a huge stimulus to develop new *in vitro*
 techniques to replace animal testing for both the scientific and
 industrial community (Goldberg and Hartung, 2006).

Required properties of *in vitro* sensitization models

There are a number of considerations which should be taken into
 account when developing an *in vitro* sensitization model. In all cases,
 for an *in vitro* model to replace an animal model it should be able
 to distinguish a sensitizer from a non-sensitizer to the same degree
 as the current animal models. However, the most important aspect
 in *in vitro* assay development is that the assay correlates to the
 human experience. Indeed, here is still room for improvement con-
 sidering that in the current animal models for example the irritant
 non-sensitizer SDS scores falsely as a sensitizer in the LLNA.
 Currently it is thought that no single assay will meet these require-
 ments and that a battery of assays should be developed which will
 be used in a tiered manner. For example epidermal penetration, DC
 maturation, DC migration and T cell priming are all aspects to be
 considered when developing *in vitro* assays. Therefore the tiered
 battery of assays is expected to be composed of not only mono-
 culture assays but also co-culture assays containing different cell
 types. If the models are to be used for regulatory purposes, they
 need to be validated i.e. their relevance and reliability should be
 established following internationally recognised procedures and
 according to human clinical data (OECD, 2005). For industrial
 screening, the manufacture and assay design should be relatively
 medium/high throughput in order to enable screening of potential
 sensitizers in a dose response manner.

Table 1Chemicals tested in *in vitro* assays listed according to their sensitizing capacity in LLNA

Abbreviation	Chemical
Extreme	
1-BA	1-Benzoylacetone ¹
BQ	<i>p</i> -Benzoquinone ²
DNCB	Dinitrochlorobenzene ³
DNFB	Dinitrofluorobenzene ⁴
K ₂ Cr ₂ O ₇	Potassium dichromate ⁵
MCI/MI	Methylchloroisothiazolinone/methylisothiazolinone ²
(NH ₄) ₂ [PtCl ₄]	Ammonium tetrachloroplatinate ^{20, *}
Oxa	Oxazolone ²
Strong	
AHT	4-amino-2-hydroxytoluene ⁶
4-AP	4-Aminophenol ⁵
BB	Bandrowski's base ⁷
BP	Balsam of Peru ⁸
BPX	Benzoyl peroxide ³
DNBS	Dinitrobenzenesulfonic acid ⁹
FA	Formaldehyde ⁵
GA	Glutaraldehyde ⁵
HE-pPDA	Hydroxyethyl- <i>p</i> -phenylenediamine ¹⁰
HPMA	2-hydroxypropyl methacrylate ^{11, *}
HEMA	2-hydroxyethyl methacrylate ^{11, *}
HQ	Hydroquinone ²
MDBGN	Methyldibromoglutaronitrile ⁷
MnCl ₂	Manganese dichloride ¹²
NiCl ₂	Nickel dichloride ¹³
PAN	Phthalic anhydride ³
PG	Propyl gallate ¹⁴
pPDA	<i>p</i> -Phenylenediamine ⁵
pTD	<i>p</i> -Toluylenediamine ¹⁰
TMA	Trimellitic anhydride ³
TMS	Thimerosal ¹⁵
TNBS	2,4,6-Trinitrobenzene sulfonic acid ¹⁰
TNCB	2,4,6-Trinitrochlorobenzene ¹⁶
Moderate	
AMP	2-amino-5-methylphenol ⁶
CNM	Cinnamaldehyde ⁵
CoCl ₂	Cobalt dichloride ¹²
CuSO ₄	Copper sulphate ¹²
DEM	Diethylmaleate ³
ED	Ethylene diamine ⁵
HCNM	Hexylcinnamaldehyde ³
IE	Isoeugenol ⁵
MAP	3-methyl-4-aminophenol ⁶
EGDMA	Ethyleneglycol dimethacrylate ^{11, *}
MBT	2-Mercaptobenzothiazole ²
MN	Methyl-2-nonynoate ⁵
Na ₂ PtCl ₄	Sodium tetrachloroplatinate ¹⁵
NiSO ₄	Nickel sulphate ¹⁷
PAA	Phenylacetaldehyde ⁵
RC	Resorcinol ¹⁸
TMTD	Tetramethylthiuram disulfide ⁵
ZnCl ₂	Zinc dichloride ¹²
Weak	
ANL	Anilin ⁵
CT	Citral ⁵
CTN	Citronellal ¹⁹
CuCl ₂	Copper chloride ²⁰
DCNB	Dichloronitrobenzene ²¹
EU	Eugenol ⁵
GE	Geraniol ⁵
HCT	Hydroxycitronellal ⁵
IU	Imidazolidinylurea ⁵
LI	Lilial ²
MDI	Diphenylmethane diisocyanate ²²
MMA	Methyl methacrylate ^{11, *}
PenG	Penicillin G ⁵
Py	Pyridine ⁵
Non-classified Sensitizers	
ASC	Ascaridol
AHCP	Ammonium hexachloroplatinate
BNP	2-bromo-2-nitropropane-1,3-diol
CdSO ₄	Cadmium sulphate
CLP	Chlorpromazine hydrochloride
CoSO ₄	Cobalt sulphate

Table 1 (continued)

Abbreviation	Chemical
CrCl ₃	Chromium trichloride
Derp 1	<i>Dermatophagoides pteronissinus</i>
DU	Diazolidinyl urea
HgCl ₂	Dichloromercury
PdCl ₂	Palladium dichloride
SnCl ₂	Stannous dichloride
Non-sensitizers	
AA	Acetanilide ²³
BA	Benzoic acid ²⁴
1-BB	1-Bromobutane ⁵
BC	Benzalkonium chloride ¹
BS	Benzalkonium sulphate ²⁵
BZC	Benzocaine ⁵
CTAB	Hexadecyltrimethylammonium bromide ⁶
CM	Coumarin ⁵
DEP	Diethyl phthalate ⁵
DMSO	Dimethyl sulfoxide ^{5, \$}
ETH	Ethanol ²⁶
GC	Glycerol ⁵
HBA	4-Hydroxybenzoic acid ⁵
HPM	2-Hydroxypropyl methacrylate ⁵
LA	Lactic acid ⁵
MC	6-Methylcoumarin ⁵
MP	Methyl paraben ⁵
MS	Methyl salicylate ⁵
pABA	<i>p</i> -Aminobenzoic acid ²⁷
PHE	Phenol ²⁸
PROP	Isopropanol ⁵
SA	Salicylic acid ⁵
SDS	Sodium dodecyl sulfate ^{5, \$}
Triton X	Octoxynol ^{29, \$}
Tween 80	Tween 80 ³⁰
VA	Vanillin ⁵

Information on LLNA is derived from the following references as indicated in superscript after chemical name: 1. Nukada et al. (2008); 2. Roberts et al. (2007b); 3. Kimber et al. (2003); 4. Basketter et al. (1997); 5. Gerberick et al. (2005); 6. Python et al. (2007); 7. Basketter et al. (2007a); 8. Staquet et al. (2004); 9. Ryan et al. (2002); 10. Aeby et al. (2004); 11. Rustemeyer et al. (2003); 12. Ikarashi et al. (1992); 13. De Smedt et al. (2005); 14. Sakaguchi et al. (2008); 15. Bocchietto et al. (2007); 16. Kimber et al. (1989); 17. Griem et al. (2003); 18. Basketter et al. (2007b); 19. Rougier et al. (2000); 20. Basketter and Scholes (1992); 21. Basketter et al. (1996); 22. Selgrade et al. (2006); 23. Gerberick et al. (2001); 24. Coutant et al. (1999); 25. Verheyen et al. (2005); 26. Betts et al. (2007); 27. Roberts et al. (2007a); 28. Mitjans et al. (2008); 29. Montelius et al. (1998); 30. Sakaguchi et al. (2006). Classified according to guinea pig test (*); classified from human patch test (#); false positive in LLNA (\$).

In this review we concentrate on the status of DC assays based on altered biomarker expression occurring after allergen exposure. There are currently two different sources of DC for assay development. These are DC derived from fresh blood cell precursors and DC-like cell lines. Whereas DC derived from fresh blood cell precursors are more physiologically relevant than DC-like cell lines, their implementation is limited by i) the logistics involved in supplying fresh blood to the cell culture lab, ii) ethical issues and iii) donor variation. Cell lines have the potential to provide large amounts of readily available DC-like cells without donor variation. However, as already mentioned, cell lines are clearly less physiologically relevant than donor derived DC. It should be noted though that since the major goal of an *in vitro* assay is to correctly distinguish a sensitizer from a non-sensitizer, the type of cell used to accomplish this goal is not as important as the reliability of the outcome of the assay. The outcome of the assay is dependent on the type of cell used and also the biomarker analyzed. Current assay development makes use of test panels of chemicals consisting of extreme, strong, intermediate and weak sensitizers and non-sensitizers all classified according to the LLNA in order to correlate the *in vitro* data with known *in vivo* data (Table 1). In this review, first the different types of cells used in *in vitro* assay development will be discussed and then the biomarkers will be discussed.

Current *in vitro* DC models

DC derived from fresh blood cell progenitors

A number of reports describe the potential of DC derived from blood cell progenitors to distinguish known sensitizers from non-sensitizers. The two commonly used sources of fresh blood cells are CD14⁺ monocytes obtained from peripheral blood and CD34⁺ haematopoietic progenitor cells (HPC) obtained from cord blood, peripheral blood or bone marrow (Meierhoff et al., 1998). Of the two, CD14⁺ monocyte derived DC (moDC) have been most extensively studied. This is probably related to the large quantity and availability of the cells to the research laboratories. However for both cell types, the logistics of a continuous supply of fresh blood to the laboratory, complex and time-consuming protocols to establish DC cultures and variability between donors and in culture conditions make it difficult to standardize these models. Furthermore, when obtaining cells from blood banks, pooled material from various donors is usually supplied.

A large number of moDC can be obtained by culturing monocytes in the presence of GM-CSF and IL-4 for approximately 6 days (Sallusto and Lanzavecchia, 1994). These moDC express a typical immature DC phenotype. They down-regulate their CD14 expression and increase their expression of CD1a, MHC class II, CD40, CD80 and CD86 upon differentiation (Meierhoff et al., 1998). Differentiating monocytes in the presence of TGF- β 1 as well as GM-CSF and IL-4, results in DC expressing Langerin which are more characteristic of LC (Ito et al., 1999). These cells are then referred to as monocyte derived LC (moLC).

HPC are able to differentiate into many different cell types according to the specific stimuli given. For example, HPC can differentiate into monocytes (in the presence of GM-CSF and TNF- α) and further into macrophages (with additional M-CSF), into interstitial DC (with additional IL-4) or LC (with additional TGF- β) (Bernhard et al., 1995; Caux et al., 1996; Herbst et al., 1998; Caux et al., 1999). For *in vitro* assays, DC have been more extensively studied than their

Table 3

Monocyte derived dendritic cells/Langerhans cells* and most frequently reported biomarkers

Chemical	HLA-DR	CXCL8	CD40	CD54	CD80	CD83	CD86	cFms	Reference ^e	
Sensitizer										
DNCB ^a	+7	+3	= ³ /+	= ² /+5	= ² /+3	= ³ /+4	+14	+	1–14*	
DNFB ^a	=/+			+	+		=/+	+	10, 19	
K ₂ Cr ₂ O ₇ ^a		=				=	=/+		5, 10	
MCl/MI ^a	=/+		=	= ²	=	=	= ²		6*, 19	
Oxa ^a	=						=	n	10	
4-AP ^b			+	+			+		7	
BB ^b	+		=		=	=	=/+3		3, 15–17	
BP ^b	+		=	=			=		6*	
DNBS ^b							+		15	
FA ^b							=		9	
HE-pPDA ^b							+		18	
HEMA ^b					+	+	+		14	
HPMA ^b					+	+	+		14	
HQ ^b							+		15	
MnCl ₂ ^b	+2		=	+	=		+3	+	1, 9, 10	
NiCl ₂ ^b	+3	+2	= ²	=/+2	= ²	+2	+5	+	1, 8–10*, 12	
PG ^b							+		15	
pPDA ^b		+	+		=/+	= ²	=/+3		5, 11, 15, 16	
pTD ^b							+		18	
TMS ^b					=		+2		9, 11	
TNBS ^b	=/+2			+		= ²	+4		2, 4, 18, 20	
TNCB ^b	=/+		=	=	=		=/+	n	1, 10	
CNM ^c			=	+		=			6*	
CLP ^c	+		+	+			+		7	
CoCl ₂ ^c	+	+	=			+	= ²	+	1, 5, 10	
CuSO ₄ ^c		+				+	+		5	
HCNM ^c	+2			+			+2		4, 20	
Na ₂ PtCl ₆ ^c					=		+		11	
NiSO ₄ ^c	+7	+3	+3	+3	+	=/+	+8		4–7*, 15, 17, 20–22	
ZnCl ₂ ^c	= ²		=	=	=		= ³	n	1, 9, 10	
EGDMA ^c					+	+	+		14	
DCNB ^d	=		=	=			=		7	
CuCl ₂ ^d	=		=	=	=		=		1	
EU ^d	=/+			+			+2		4, 20	
ASC				+			+		13	
CdSO ₄ ^d	+						+	n	10	
MMA ^d					+	+	+		14	
CrCl ₃		+					=	+	5	
HgCl ₂	=						=	n	10	
PdCl ₂		+					=		5	
SnCl ₂	+						+	n	10	
Non-sensitizer										
BA ^{ns}	=		=	=			= ²		7, 15	
BC ^{ns}	=	–	=	=	=	=	=/+		1, 2, 5, 9, 15	
CM ^{ns}						+	+		3	
DMSO ^{ns}	= ² /+		= ²	= ³	=	–	–/+3		1, 4, 5, 7, 9	
PROP ^{ns}			=				=		5	
SDS ^{ns}	=6	–/=	= ³	= ⁴ /+	= ³	= ³	= ¹⁰ /		1, 2, 4–7*, 9, 11, 15, 18–20	

Most frequently reported changes in biomarker expression after exposure to sensitizers and non-sensitizers. Up-regulation (+), down-regulation (–), non-significant (=) changes in marker expression after chemical exposure and marker not detected (n) are shown along with the number of independent citations if this is more than 1. For example: +2 indicates 2 independent citations reporting an increase in biomarker expression. Chemicals are classified according to the LLNA in extreme^a, strong^b, moderate^c, weak^d and non sensitizer^{ns}. References^e: 1. Aiba et al. (1997); 2. Straube et al. (2005); 3. Jugde et al. (2005); 4. Tuschl and Kovac (2001); 5. Toebak et al. (2006b); 6. Staquet et al. (2004); 7. Coutant et al. (1999); 8. Aiba et al. (2000); 9. Mizuashi et al. (2005); 10. Manome et al. (1999); 11. Bocchietto et al. (2007); 12. Aiba et al. (2003); 13. Tietze and Blomeke (2008); 14. Rustemeyer et al. (2003); 15. Hulet et al. (2002); 16. Coulter et al. (2007); 17. Szameit et al. (2008); 18. Aebly et al. (2004); 19. Hulet et al. (2005); 20. De Smedt et al. (2002); 21. Arrighi et al. (2001); 22. Toebak et al. (2008). Refs. 6* and 8* = monocyte derived Langerhans cells (moLC) were exposed to chemical rather than moDC.

LC counterpart. The immature DC and LC have been shown to exhibit phenotypic changes associated with DC maturation upon exposure to a sensitizer thus making them candidates for *in vitro* assay development (Tables 2 and 3).

Table 2
CD34⁺ derived dendritic cells/Langerhans cells* and most frequently reported biomarkers

Chemical	HLA-DR	CD83	CD86	IL-1 β	IL-6	CXCL8	IL-12	TNF- α	References ^e
Sensitizer									
DNCB ^a	=/+2	+3	+3	+	=/+2	+2	= ²		1–4
DNFB ^a	=	=	+	=	=		=		1
K ₂ Cr ₂ O ₇ ^a	+	=	=	=	=	–			1
Oxa ^a	=	+	=	=	–/+	+	=	+	1, 2
BB ^b	+	+	+						5*
DNBS ^b	=	=	+	=			=		1
NiCl ₂ ^b	+2	+2	+2	n	+2		= ²	= ²	1, 6
pPDA ^b	=	=	=						5*
TNBS ^b	+2	+2	+2	+	=		=	–	1, 7
HCNM ^c	=/+	= ²	= ²	+	=		=	+	1, 7
NiSO ₄ ^c	+3	+3	+3	+2	+2	+2	+		2, 3, 4, 7
CTN ^d	=	=	=						5*
EU ^d	= ²	=/+	+2	+2	–2	+	=	–	1, 2, 7
Non-sensitizer									
BC ^{ns}	=	=	=	=	=		=		1
BS ^{ns}				=	=				2
CM ^{ns}	=	=	=				=		5*
DMSO ^{ns}	=	=	=		+		=		4
SDS ^{ns}	=4	=4	=4	n/=+	=3	+	= ²	+	1, 2, 5*, 6, 7

Most frequently reported changes in biomarker expression after exposure to sensitizers and non-sensitizers. Up-regulation (+), down-regulation (–), non-significant (=) changes in marker expression and marker not detected (n) after chemical exposure are shown along with the number of independent citations if more than 1. For example: +2 indicates 2 independent citations reporting an increase in biomarker expression. Chemicals are classified according to the LLNA in extreme^a, strong^b, moderate^c, weak^d and non sensitizer^{ns}. References^e: 1. De Smedt et al. (2005); 2. Verheyen et al. (2005); 3. Boisleve et al. (2004); 4. Ade et al. (2007); 5. Rougier et al. (2000); 6. De Smedt et al. (2001); 7. De Smedt et al. (2002). Ref. 5* = CD34⁺ derived Langerhans cell.

Table 4

THP-1 cell line and most frequently reported biomarkers

Chemical	CD40	CD54	CD80	CD83	CD86	CXCL8	MIP-1 β	TNF- α	p38	References
<i>Sensitizer</i>										
1-BA ^a		+			=	+				1, 2
BQ ^a		+			+					1
DNCB ^a	+ ³	=/+ ⁹	+ ²	+	+ ¹³	+ ⁴	+ ²	+ ³	+ ²	1–15
DNFB ^a							+			10
(NH ₄) ₂ [PtCl ₄] ^a		+ ³			=/+ ⁴		+			1, 3–6
K ₂ Cr ₂ O ₇ ^b		+			=		+			1, 10
MCI/MI ^a		=			=		+			2, 10, 16
BPX ^b						+				2
FA ^b	+	= ² /+ ³		+	=/+ ⁴	=	+	+ ²		1, 3, 5, 7, 11, 16
GA ^b		+			+	+				1, 2
HQ ^b	+	= ²		+	+ ²	+	+	+		1, 7, 10
MDBGN ^b		+			+					16
PAN ^b						=				2
PG ^b	+	+ ³		+	= ²	+		=/+		1, 7, 11
pPDA ^b		=/+ ³	=		+ ⁷	+	=		+	1, 3–6, 8, 13, 14
TMA ^b		+			=	=				14
TMS ^b			=		+					13
CNM ^c		+ ²			+ ²	+			+	1, 14
CoSO ₄ ^c		+ ²			= ³ /+		+			3–6
DEM ^c						+				2
ED ^c		=			+	=				1, 2
HCNM ^c		=			=	+	+			1, 2, 10
IE ^c		=			=					1
MBT ^c		+ ³			= ³ /+ ²	+	+			1–6
MN ^c						+				2
Na ₂ PtCl ₄ ^c			=		+					13
NaSO ₄ ^c	+ ³	+ ⁷		+	= ³ /+ ⁵	+ ⁴	+ ²	+ ³	+ ²	1–8, 10–12, 14
PAA ^c	+	+		+	+ ²	+		+		1, 7
RC ^c		+			+					1
TMTD ^c		=			+	+			+	14
ANL ^d		=			=	+				2, 3
CT ^d					=	+	=			2, 5
CuCl ₂ ^d		=			=					3
EU ^d	+	+ ³		=	+ ³	=/+	+	= ²		1, 2, 4, 7, 10, 11
GE ^d					=					2
HCT ^d	+	+		+	=/+	+	+	+		5, 7
IU ^d		+			+					16
LI ^d		+			=					1
MDI ^d		+			=	+			+	14
PenG ^d						+				14
Py ^d		+			+					1
ASC		+			+					15
AHPT		+			+		+		+	14
BNP		+			+					16
Derp1						+			+	17
DU		+			=					16
HgCl ₂					+		+			5
<i>Non-sensitizer</i>										
AA ^{ns}		=			=	=				1, 2
1-BB ^{ns}						+				2
BC ^{ns}		=			= ²	=	= ²			1, 2, 5
BZC ^{ns}							+			10
DEP ^{ns}						+				2
DMSO ^{ns}	=	= ³	=	=	= ⁵	=	= ²	=		3–7, 10
GC ^{ns}		=			=					1
HBA ^{ns}		= ²			= ²					1, 16
HPM ^{ns}						=				2
LA ^{ns}	=	=		=	= ²	=	=	=		1, 7, 10
MC ^{ns}						=				2
MP ^{ns}	=	= ³		=	= ³	=		=		1, 7, 16
MS ^{ns}		=			= ³		= ²			3–5, 10
PHE ^{ns}		+			=	=				14
PROP ^{ns}							=			10
SA ^{ns}		=			=	=	=			10, 14
SDS ^{ns}	= ²	= ⁷ /+	=	=	= ¹¹	= ³	=/+	= ²		1, 3–11, 13, 14
TritonX ^{ns}					=		=			5
Tween80 ^{ns}		= ²			= ²					3, 6
VA ^{ns}		=			=	=				1, 2

Most frequently reported changes in biomarker expression after exposure to sensitizers and non-sensitizers. Up-regulation (+), down-regulation (–), non-significant (=) changes in marker expression after chemical exposure are shown along with the number of independent citations if more than 1. For example: +² indicates 2 independent citations reporting an increase in biomarker expression. Chemicals are classified according to the LLNA in extreme^a, strong^b, moderate^c, weak^d and non sensitizer^{ns}. References: 1. Sakaguchi et al. (2008); 2. Nukada et al. (2008); 3. Yoshida et al. (2003); 4. Ashikaga et al. (2002); 5. Hirota and Moro (2006); 6. Sakaguchi et al. (2006); 7. Miyazawa et al. (2007); 8. Ashikaga et al. (2006); 9. Azam et al. (2006); 10. Lim et al. (2008); 11. Miyazawa et al. (2008b); 12. Miyazawa et al. (2008a); 13. Bocchietto et al. (2007); 14. Mitjans et al. (2008); 15. Tietze and Blomeke (2008); 16. Sakaguchi et al. (2007); 17. Lee et al. (2008).

DC-like cell lines

The use of human DC-like cell lines to predict a potential sensitizer from a non-sensitizer has many advantages when compared to DC derived from fresh blood cells. Importantly, no donor to donor variation is involved since a cell line originates from a single donor. Since most cell lines are available via cell and tissue culture companies, the cells are readily available with a standard culture protocol. This means that large numbers of cells can be grown via standardized conditions in a short time and that experiments can be easily planned. In this is not the case when the laboratory is dependent on an often unreliable and irregular source of fresh blood for its source of DC. Therefore implementing DC-like cell lines into cell based assays is much simpler than implementing cell assays which are dependent of fresh blood. However, it should be noted that it is possible to introduce variations within a cell line at high passage numbers and when culturing under slightly different conditions in different laboratories. In order to be a suitable candidate for an *in vitro* assay a DC-like cell line should exhibit a measurable phenotypic change upon exposure to a sensitizer, preferably which is characteristic of DC maturation.

Whilst a DC cell line which physiologically represents its *in vivo* counterpart is in many ways preferred it is not an essential criteria for the assay. According to the literature, human monocytic leukemia cell line (THP-1), human histiocytic lymphoma cell line (U-937), CD34⁺ human acute myeloid leukemia cell line (MUTZ-3), human bone marrow-derived acute myelogenous leukemia cell line (KG-1), human acute pro-myeloid leukemia cell line (HL-60), and human erythroleukemia cell line (K562) are cell lines that have been used in studies aiming to distinguish known sensitizers from non-sensitizers using a well defined test panel of chemicals. The characteristics of each of these cell lines are described below.

Human monocytic leukemia cell line (THP-1)

THP-1 cells are phagocytic cells which originate from the peripheral blood of a male with acute monocytic leukemia (Tsuchiya et al., 1980; Tsuchiya et al., 1982). In their naïve undifferentiated form the cells express typical monocytic surface markers such as CD58⁺, CD86^{low} and CD40^{low}. Markers such as CD54, CD83 and CD1a are almost negative (Miyazawa et al., 2007). After culture with GM-CSF, IL-4, TNF-α, some phenotypic changes can be induced which are characteristic of a DC-like phenotype (Yoshida et al., 2003; Santegoets et al., 2008). They then express high levels of CD54 and low levels of CD1a, HLA-DR, CD80, and CD86. However, since the naïve THP-1 has performed better in assay development until date compared to the differentiated THP-1 most studies use the naïve THP-1 (Yoshida et al., 2003) (see Table 4). The cells are easy to culture and to amplify and therefore have been used in many promising studies.

Human histiocytic lymphoma cell line (U-937)

The U-937 cell line was established in 1974 and originates from a histiocytic lymphoma patient. U-937 cells exhibit monocytic characteristics by displaying monoblast morphology, lysozyme production and esterase activity (Sundstrom and Nilsson, 1976). They do not display phagocytosis activity. They express basal levels of CD14, CD54 and CD86, although HLA-DR is undetectable (Azam et al., 2006). Upon culture with either G-CSF, GM-CSF, conditioned medium from CM5637 cell line or IL-4, U-937 cells decrease their CD14 expression and increase some markers characteristic for differentiated cells although CD1a is absent (Koss et al., 1996; Santegoets et al., 2008). As with THP-1 cells, U-937 have been described mainly in their naïve undifferentiated state in *in vitro* assay development (see Table 5).

CD34⁺ human acute myeloid leukemia cell line (MUTZ-3)

MUTZ-3 is a very promising cell line for incorporation into an *in vitro* assay since compared to all the cell lines studied until now,

Table 5
U-937 cell line and most frequently reported biomarkers

Chemical	CD54	CD86	IL-1β	CXCL8	References
Sensitizers					
DNCB ^a	= ²	=/+ ²	+	+	1–3
(NH ₄) ₂ [PtCl ₄] ^a	=	+			1
Oxa ^a		=	=	+	3
AHT ^b		+	+	+	3
pPDA ^b	=	+			1
HE-pPDA ^b		+	=	=	3
pTD ^b		+	+	+	3
TNBS ^b		+	+	+	3
AMP ^c		+	+	+	3
CoSO ₄ ^c	=	=			1
HCNM ^c		=	=	=	3
MAP ^c		+	+	+	3
MBT ^c	=	=/+	+	+	1, 3
NiSO ₄ ^c	=	=/+	=	+	1, 3
CT ^d		+	=	=	3
EU ^d		+	+	+	3
CTAB		=	=	+	3
Non-sensitizers					
DMSO ^{ns}	=	= ²	=	=	1, 3
MS ^{ns}		=	=	+	1
SDS ^{ns}	–/=	=/+ ²	=	=	1–3
Tween80 ^{ns}	=	=			1

Most frequently reported changes in biomarker expression after exposure to sensitizers and non-sensitizers. Up-regulation (+), down-regulation (–), non-significant (=) changes in marker expression after chemical exposure are shown along with the number of independent citations if more than 1. For example: +² indicates 2 independent citations reporting an increase in biomarker expression. Chemicals are classified according to the LLNA in extreme^a, strong^b, moderate^c, weak^d and non sensitizer^{ns}. References: 1. Sakaguchi et al. (2006); 2. Azam et al. (2006); 3. Python et al. (2007).

MUTZ-3 most physiologically represents its *in vivo* counterpart. The MUTZ-3 progenitor cell line consists of a heterogeneous cell population. Small CD34(+)CD14(–)CD11b(–) progenitors are the proliferating population in the cell line. These are able to differentiate through a CD34(–)CD14(–)CD11b(+) stage to give rise to a morphologically large, non-proliferating CD14(+)CD11b(hi) progeny. All 3 subsets are CD1a, CD80 and CD83 negative (Masterson et al., 2002; Azam et al., 2006). MUTZ-3 progenitor cells are unique in that their survival and proliferation are cytokine dependent. Importantly, the CD14(+)CD11b(hi) progeny can be skewed in a cytokine dependent manner similar to HPC into either interstitial DC (MUTZ-DC) expressing CD1a and DC-SIGN, or into Langerhans cells (MUTZ-LC) expressing CD1a, Langerin and containing Birbeck granules (Masterson et al., 2002; Larsson et al., 2006; Santegoets et al., 2008). A cytokine cocktail containing GM-CSF, TNF-α and IL-4 generates MUTZ-DC whereas a cytokine cocktail containing GM-CSF, TNF-α and TGF-β generates MUTZ-LC. Upon maturation MUTZ-DC and MUTZ-LC increase expression of typical markers for DC maturation such as CD80, CD83, and CD86. The MUTZ-DC but not the MUTZ-LC have been shown to stimulate in an allogeneic MLR in a similar manner to moDCs (Santegoets et al., 2006). However, both MUTZ-DC and MUTZ-LC only secrete very low levels of IL-12 indicating a clear difference from non-malignant native DC (Santegoets et al., 2008). Until now only a single study describing the MUTZ-3 progenitor cell in *in vitro* assay development has been described (Azam et al., 2006) (see Table 6).

Human bone marrow-derived acute myelogenous leukemia cell line (KG-1)

The KG-1 cell line is derived from bone marrow and is a human cell line that maintains the granulocytic characteristics of acute myelogenous leukemia cells (Koeffler and Golde, 1978; Koeffler and Golde, 1980). Culture in the presence of TNF-α can induce some differentiation but only treatment with phorbol-myristil-acetate and ionomycin (with or without prior culture in GM-CSF and IL-4) can induce some

Table 6

Infrequently reported cell lines (MUTZ-3, KG-1, HL-60, and K562) and biomarkers

Chemical	HLA-DR	CD40	CD54	CD80	CD86	B7-H1	B7-H2	B7-DC	References
MUTZ-3									
Sensitizer									
DNCB ^a	=	=	+		+	=	=	=	1
HQ ^b	=	+	+		+	+	=	=	1
HCNM ^c	–	+	+		+	+	=	=	1
EU ^d	–	+	+		+	+	=	=	1
Non-sensitizer									
BZC ^{ns}	–	–			+	–	=	=	1
pABA ^{ns}	=	=	=		=	=	=	=	1
SDS ^{ns}	=	–	=		–	+	=	=	1
KG-1									
Sensitizer									
DNCB ^a	=		+ ²			=/+			1, 2
DNFB ^a	=		=	=					3
MCI/MI ^a	=		=	=		+			3
Non-sensitizer									
DMSO ^{ns}			=			=			2
SDS ^{ns}	= ²		= ²	=		= ²			1, 3
HL-60									
Sensitizer									
DNCB ^a	0		+		+				1
Non-sensitizer									
SDS ^{ns}	0		=		+				1
K562									
Sensitizer									
DNCB ^a	0		=		0				1
Non-sensitizer									
SDS ^{ns}	0		=		0				1

Most frequently reported changes in biomarker expression after exposure to sensitizers and non-sensitizers. Up-regulation (+), down-regulation (–), non-significant (=) changes in marker expression after chemical exposure are shown along with the number of independent citations if more than 1. For example: +² indicates 2 independent citations reporting an increase in biomarker expression. Chemicals are classified according to the LLNA in extreme^a, strong^b, moderate^c, weak^d and non-sensitizer^{ns}.

References: 1. Azam et al. (2006); 2. Yoshida et al. (2003); 3. Hulet et al. (2002).

morphological and phenotypic changes consistent with DC-like maturation e.g.: low CD1a expression, low levels of macromolecular endocytosis, and limited ability to stimulate in allogeneic MLR compared with moDCs. Therefore KG-1 cells can differentiate *in vitro* into cells with DC-like functional characteristics under the influence of strong inducers of cellular activation, but they lack the potency of mature DCs in many aspects of professional antigen presenting cells (Hulet et al., 2001; Azam et al., 2006; Teobald et al., 2008). However, differentiation is accompanied by the distinct up-regulation of the differentiation marker CD83, indicating simultaneous maturation together with differentiation (Santegoets et al., 2008). Both undifferentiated KG-1 (Azam et al., 2006; Yoshida et al., 2003) and differentiated KG-1 (Hulet et al., 2002) cells have been exposed to limited test panels of chemicals, however the data available so far is so limited that it cannot be concluded yet in which form KG-1 may potentially perform best in *in vitro* assays (see Table 6).

Human acute pro-myeloid leukemia cell line (HL-60) and human erythroleukemia cell line (K562)

The HL-60 cell line is derived from the fresh blood of an acute promyelocytic leukemia patient and is a multipotent cell line capable of differentiating into granulocyte-, monocyte-macrophage- or eosinophil-like cells. The K562 cell line is another multipotent cell line capable of differentiating into megakaryocytic-, erythroid- and monocytic-like cells. Phenotypic analysis has shown that both HL-60 and K562 cells do not express HLA-DR. K562 cells do not express CD86 in contrast to HL-60. CD54 is present in both cell lines, however CD54 is higher on K562 cells than on HL-60 cells (Azam et al., 2006). Both HL-60 and K562 have a very limited capacity to differentiate into DC-like cells since they fail to express CD1a upon differentiation with GM-CSF, IL-4 and TNF- α (Santegoets et al., 2008). As with KG-1, the number of studies performed with either HL-60

or K562 is so limited that it is not possible to draw any conclusions about their suitability for cell based assays aimed at distinguishing sensitizers from non-sensitizers (Azam et al., 2006) (see Table 6). The limited number of reports published may be due to either a lack of extensive investigation or due to unsatisfactory results not considered worthy of publishing.

Biomarkers used in *in vitro* sensitization models

Equally important to the type of cell used to develop an *in vitro* sensitization assay is the type of biomarker used as readout. For an overview of the biomarkers described in the literature, see Tables 2–6. In order to gain insight into the ability of *in vitro* assays to determine the potency of the sensitizer, the chemicals described in the tables have also been ranked as extreme, strong, moderate and weak sensitizers in accordance to the classification system obtained from the LLNA (Table 1). Until date, the cell surface marker CD86 is the most extensively studied biomarker.

Surface markers

Since DC maturation upon exposure to a sensitizer is accompanied by changes in surface marker expression such surface markers are potential biomarkers in an *in vitro* assay. The most frequently tested markers are CD40, CD54, CD80, CD83, CD86, and HLA-DR as shown in Tables 2–6. These are all surface markers which have been described to be up-regulated upon DC maturation. Of all the biomarkers tested until now, increased CD86 expression has been the most extensively studied and is the most promising biomarker to date. A single study describes the exposure of THP-1 to 29 chemicals (Sakaguchi et al., 2008) (Table 4). However this single marker is far from adequate for *in vitro* testing since increases in

CD86 expression are slight. Also, not all sensitizers result in an increase in CD86 expression, and changes in CD86 expression do not permit strong, intermediate and weak sensitizers to be distinguished from each other. The assay was slightly improved by performing a combined analysis of CD86 with CD54.

Amongst the infrequently tested surface markers are CD1a, Langerin and E-cadherin. These three markers were down-regulated in CD34⁺DC upon exposure to a limited test panel of chemicals and in this way their regulation mimics the *in vivo* situation upon DC maturation (Boislevé et al., 2004). Other less frequently tested surface markers which were tested in moDC include MHCI, MHCII, CLA, CD25, CD29, CD44, CD44v6, CD49e, CD49f, CD49g, AQP3, P-glycoprotein and lung resistant protein (Aiba et al., 2000; Rustemeyer et al., 2003; Aebly et al., 2004). Changes in HLA-DR and CD1a expression were analyzed in THP-1 cells after exposure to a restricted panel of chemicals (Yoshida et al., 2003; Azam et al., 2006; Miyazawa et al., 2007) and changes in HLA-DR, B7-H1, B7-H2 and B7-DC were analyzed in MUTZ-3 cells also after exposure to a restricted panel of chemicals (Azam et al., 2006). However, in all cases, due to the limited number of chemicals tested, no strong conclusions can be drawn with regard to these infrequently tested potential biomarkers and more extended studies are required.

Chemokines/cytokines and their receptors

In addition to the changes in expression of the surface markers described above, upon DC maturation changes in cytokine and chemokine secretion and receptor expression occur in order to permit migration of DC out of the skin and to initiate their journey towards the draining lymph nodes. It may be possible to exploit this knowledge and to identify novel biomarkers for *in vitro* assays. This is indeed the approach of a number of studies.

CXCL8 (also known as IL-8) is one of the most promising recently identified biomarkers for distinguishing sensitizers from non-sensitizers (Toebak et al., 2006b; Python et al., 2007; Mitjans et al., 2008; Miyazawa et al., 2008b; Nukada et al., 2008). Studies using moDC, CD34⁺DC and THP-1 cells indicate that increased CXCL8 secretion or transcription may be just as sensitive and in some cases a more sensitive biomarker than CD86. Also quantification of CXCL8 can be performed by Enzyme Linked Immunosorbent Assay (ELISA). This technique is by far simpler and of higher throughput than the difficult to quantify flow cytometry technique currently used for CD86. Therefore the suitability of CXCL8 as a biomarker warrants further investigation in extended studies. Additionally chemokines CCL2, CCL3, CCL3L1, CCL4, and the receptor CCR7 expression have been studied by different research groups after CD34⁺ DC were exposed to a limited panel of sensitizers (DNFB, Oxazolone, NiSO₄, and Eugenol) and non-sensitizers (BS, DMSO, SDS) (Verheyen et al., 2005; Schoeters et al., 2006; Ade et al., 2007; Schoeters et al., 2007). In general, an increase in chemokine secretion/receptor expression was observed when sensitizers were tested but not when non-sensitizers were tested although no single chemokine ligand/receptor was conclusive and results were sometimes contradictory between the studies. Similarly other studies using moDC have described chemokines CCL3, CCL4, CCL5, CCL11, CCL17, CCL18, CCL19, CCL20, CCL22, CXCL2, CXCL12, and receptors CCR1, CCR2, CCR3, CCR5, CCR6, CCR7, CCR8, CXCR4, CXCR5 after chemical exposure (Aiba et al., 2000; Rustemeyer et al., 2003; Staquet et al., 2004; Jugde et al., 2005; Toebak et al., 2006b; Szameit et al., 2008). Although again due to the limited number of studies and the limited number of chemicals tested no clear conclusions can be drawn and further extended studies are required. With regard to DC-like cell lines, only a very limited number of chemokines (CXCL8, CCL2 and CCL4) have been studied and then only in THP-1 and therefore again more intensive studies are required (Lee et al., 2008).

Amongst the cytokines, IL-1 β , IL-6, IL-10, IL-12, IL-15, IL-18 and TNF- α have been most studied (Aiba et al., 1997; Coutant et al., 1999;

Manome et al., 1999; Aiba et al., 2000; Pichowski et al., 2000; Pichowski et al., 2001; Tuschl et al., 2001; De Smedt et al., 2002; Aiba et al., 2003; Aebly et al., 2004; Jugde et al., 2005; Toebak et al., 2006a; Toebak et al., 2006b; Szameit et al., 2008; Toebak et al., 2008). The majority of the studies were performed using DC derived from fresh blood cells (moDC and CD34⁺DC) with only IL-1 β and IL-6 being described in a DC-like cell line, THP-1 (Miyazawa et al., 2007). The most extensively studied cytokine in DC based assays is IL-1 β (Aiba et al., 1997; Coutant et al., 1999; Pichowski et al., 2001; De Smedt et al., 2002; Yoshida et al., 2003; Aebly et al., 2004; Boislevé et al., 2004; Schoeters et al., 2006). It should be noted that when using DC derived from fresh blood cell precursors donor variation has been reported. Exposure of moDC to allergens (e.g.: DNFB, pPDA, MCI) resulted in up-regulation of IL-1 β mRNA in only three out of eight donors (Pichowski et al., 2000; Pichowski et al., 2001). Cytokines as biomarkers however, should not be ruled out since current data is limited and more extended studies might identify a more promising cytokine biomarker.

Kinases

Signal transduction cascades precede changes in surface marker expression, and cytokine/chemokine secretion. Components of signal transduction pathways are kinases which phosphorylate and dephosphorylate a cascade of substrates in order to cause a change in the expression/secretion of a target molecule (in this case the surface marker protein, the cytokine or the chemokine). Therefore components of the signal transduction cascade may prove to be ideal novel biomarkers to distinguish sensitizers from non-sensitizers. A limited number of studies have addressed this subject and these are all directed at measuring changes in kinase expression. Only moDC (Arrighi et al., 2001; Aiba et al., 2003; Mizuashi et al., 2005; Nukada et al., 2008; Szameit et al., 2008), CD34⁺DC (Boislevé et al., 2004; Ade et al., 2007; Nukada et al., 2008) and THP-1 (Mitjans et al., 2008; Miyazawa et al., 2008a; Nukada et al., 2008) have been studied until date. P38 MAPK, a mitogen-activated protein kinase, has been identified as a promising novel biomarker in these three cell based assays. Potential other candidates are PGK, ERK, SAPK/JNK, I κ B, NF κ B, TNFRSF1A although data is very limited.

Genomics and proteomics

Genomic and proteomic studies have the potential to reveal many novel biomarkers in a DC based assay. However, a limiting factor in these studies is that they are generally restricted to only one or two test chemicals. Once a potential biomarker has been identified it needs to be further investigated in an experimental setting where the test model is exposed to an extended panel of test chemicals using quantitative biomarker analytical techniques. In this way it can be determined whether the biomarker is a representative marker for sensitization in general or only a marker for a particular type of chemical. Such a study has been described by Ryan et al. (2004) who performed microarray analysis on moDC in the presence or absence of DNFB and Schoeters et al. (2005, 2006) who performed microarray analysis for CD34⁺DC after exposure to DNFB or NiSO₄. Hereafter, custom designed arrays or quantitative PCR of selected genes may be used to distinguish sensitizers from non-sensitizers (Gildea et al., 2006; Cluzel-Tailhardat et al., 2007; Schoeters et al., 2007; Hooyberghs et al., 2008; Szameit et al., 2008). An example of such a study describes 13 target genes which were first pinpointed by microarray techniques in CD34⁺DC and which were then further screened with the aid of quantitative PCR in order to distinguish 10 sensitizers from 11 non-sensitizers with a prediction performance with a concordance of 89%, a specificity of 97% and a sensitivity of 82% using samples from 73 different donors (Hooyberghs et al., 2008). Similar approaches could be applied to

extensive proteomics in which potential targets could be further studied on for example the protein level by ELISA, western blot techniques or flow cytometry. However, until date, no such studies have been described.

Conclusion and future prospects

From this review it can be concluded that the CD86, CXCL8 and p38 MAP kinase are the most promising and extensively tested biomarkers described to date in DC based assays. It is possible that the combined analysis of these 3 biomarkers rather than analysis of a single biomarker will give even more satisfactory results. Many other biomarkers have been described but no clear conclusions can be drawn due to the sub optimal test conditions used e.g.: test panel of chemicals is too limited and/or no non-sensitizer controls. The THP-1 cell line has been most extensively studied with very promising results, although more extensive studies using U-937 or MUTZ-3 progenitors may show that these DC-like cell lines are just as good. Furthermore, current assays aim at distinguishing a sensitizer from a non-sensitizer. Only limited studies address the potency of the sensitizer i.e.: whether it is an extreme, strong, moderate or weak sensitizer (Azam et al., 2006; Nukada et al., 2008). In these studies, not all chemicals tested gave results which were in accordance with LLNA data.

Notably, when summarising the literature it can be concluded that whilst DC cell based assays look very promising (e.g.: U-937 and THP-1 assays using CD86 as biomarker) these assays will most probably not be suitable as a stand alone assay to distinguish a potential sensitizer from a non-sensitizer to the same level of accuracy and reliability as the LLNA. This is mainly due to solubility and stability issues around cell based assays in which the chemical has to be added to the cell culture medium. Vehicles such as DMSO, acetone, olive oil aid this to a certain extent but even so a relatively large number of false positives and negatives are recorded, and only minor changes (increase/decrease) in biomarker expression are observed after sensitizer exposure. Therefore fundamental mechanistic issues related to T-cell repertoire may also be involved. When investigating mechanisms involved in sensitization *in vivo* and trying to extrapolate these to *in vitro* assay development, it can be expected that a battery of assays will be required. These assays would take into account bioavailability/skin penetration; protein reactivity/binding, cross talk between skin residential cells and DC; DC maturation, DC migration and T-cell priming. A major limitation in existing cell-based *in vitro* models is that no immuno-competent skin equivalent is commercially available and very few are in development (Regnier et al., 1997). Such models would permit bioavailability/skin penetration studies and also cross talk between epidermal cells and DC to be investigated for their potential exploitation into the area of assay development. Furthermore, since skin equivalents have a barrier function (Ponec et al., 2001), water insoluble compounds can be applied topically in a similar manner to patch testing individuals *in vivo* thus solving the solubility issues around testing water insoluble compounds.

As more and more information become available on DC biology the panel of potential and novel markers is continuously expanding. Also particularly noticeable from our tables is that whereas a large number of chemicals have been investigated in *in vitro* sensitization assays, the number of different biomarkers investigated is extremely limited. This observation calls out to scientists to exploit genomic, proteomic and metabonomic technology in order to identify novel biomarkers which will in turn lead to a better understanding of mechanisms underlying skin sensitization and in turn aid the development of *in vitro* assays. From our review we can conclude that even though progress is promising, scientists will be hard pushed to have fully validated assays implemented in time to meet the up and coming European regulatory deadlines.

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References

- Ade, N., Antonios, D., Kerdine-Romer, S., Boisleve, F., Rousset, F., Pallardy, M., 2007. NF-kappaB plays a major role in the maturation of human dendritic cells induced by NiSO₄ (4) but not by DNCB. *Toxicol. Sci.* 99, 488–501.
- Aeby, P., Wyss, C., Beck, H., Griem, P., Scheffler, H., Goebel, C., 2004. Characterization of the sensitizing potential of chemicals by *in vitro* analysis of dendritic cell activation and skin penetration. *J. Invest. Dermatol.* 122, 1154–1164.
- Aiba, S., Terunuma, A., Manome, H., Tagami, H., 1997. Dendritic cells differently respond to haptens and irritants by their production of cytokines and expression of co-stimulatory molecules. *Eur. J. Immunol.* 27, 3031–3038.
- Aiba, S., Manome, H., Yoshino, Y., Tagami, H., 2000. *In vitro* treatment of human transforming growth factor-beta1-treated monocyte-derived dendritic cells with haptens can induce the phenotypic and functional changes similar to epidermal Langerhans cells in the initiation phase of allergic contact sensitivity reaction. *Immunology* 101, 68–75.
- Aiba, S., Manome, H., Nakagawa, S., Mollah, Z.U., Mizuashi, M., Ohtani, T., Yoshino, Y., Tagami, H., 2003. p38 Mitogen-activated protein kinase and extracellular signal-regulated kinases play distinct roles in the activation of dendritic cells by two representative haptens, NiCl₂ and 2,4-dinitrochlorobenzene. *J. Invest. Dermatol.* 120, 390–399.
- Antonopoulos, C., Cumberbatch, M., Mee, J.B., Dearman, R.J., Wei, X.Q., Liew, F.Y., Kimber, I., Groves, R.W., 2008. IL-18 is a key proximal mediator of contact hypersensitivity and allergen-induced Langerhans cell migration in murine epidermis. *J. Leukoc. Biol.* 83, 361–367.
- Arrighi, J.F., Rebsamen, M., Rousset, F., Kindler, V., Hauser, C., 2001. A critical role for p38 mitogen-activated protein kinase in the maturation of human blood-derived dendritic cells induced by lipopolysaccharide, TNF-alpha, and contact sensitizers. *J. Immunol.* 166, 3837–3845.
- Ashikaga, T., Hoya, M., Itagaki, H., Katsumura, Y., Aiba, S., 2002. Evaluation of CD86 expression and MHC class II molecule internalization in THP-1 human monocyte cells as predictive endpoints for contact sensitizers. *Toxicol. In Vitro* 16, 711–716.
- Ashikaga, T., Yoshida, Y., Hirota, M., Yoneyama, K., Itagaki, H., Sakaguchi, H., Miyazawa, M., Ito, Y., Suzuki, H., Toyoda, H., 2006. Development of an *in vitro* skin sensitization test using human cell lines: the human Cell Line Activation Test (h-CLAT). I. Optimization of the h-CLAT protocol. *Toxicol. In Vitro* 20, 767–773.
- Azam, P., Peiffer, J.L., Chamoussat, D., Tissier, M.H., Bonnet, P.A., Vian, L., Fabre, I., Ourlin, J.C., 2006. The cytokine-dependent MUTZ-3 cell line as an *in vitro* model for the screening of contact sensitizers. *Toxicol. Appl. Pharmacol.* 212, 14–23.
- Basketter, D.A., Cadby, P., 2004. Reproducible prediction of contact allergenic potency using the local lymph node assay. *Contact Dermatitis* 50 (1), 15–17.
- Basketter, D.A., Scholes, E.W., 1992. Comparison of the local lymph node assay with the guinea-pig maximization test for the detection of a range of contact allergens. *Food Chem. Toxicol.* 30, 65–69.
- Basketter, D.A., Scholes, E.W., Fielding, I., Dearman, R.J., Hilton, J., Kimber, I., 1996. Dichloronitrobenzene: a reappraisal of its skin sensitization potential. *Contact Dermatitis* 34, 55–58.
- Basketter, D.A., Dearman, R.J., Hilton, J., Kimber, I., 1997. Dinitrohalobenzenes: evaluation of relative skin sensitization potential using the local lymph node assay. *Contact Dermatitis* 36, 97–100.
- Basketter, D.A., Gerberick, F., Kimber, I., 2007a. The local lymph node assay and the assessment of relative potency: status of validation. *Contact Dermatitis* 57, 70–75.
- Basketter, D.A., Sanders, D., Jowsey, I.R., 2007b. The skin sensitization potential of resorcinol: experience with the local lymph node assay. *Contact Dermatitis* 56, 196–200.
- Bernhard, H., Disis, M.L., Heimfeld, S., Hand, S., Gralow, J.R., Cheever, M.A., 1995. Generation of immunostimulatory dendritic cells from human CD34+ hematopoietic progenitor cells of the bone marrow and peripheral blood. *Cancer Res.* 55, 1099–1104.
- Betts, C.J., Beresford, L., Dearman, R.J., Lalko, J., Api, A.P., Kimber, I., 2007. The use of ethanol:diethylphthalate as a vehicle for the local lymph node assay. *Contact Dermatitis* 56, 70–75.
- Bocchietto, E., Paolucci, C., Breda, D., Sabbioni, E., Burastero, S.E., 2007. Human monocytoid THP-1 cell line versus monocyte-derived human immature dendritic cells as *in vitro* models for predicting the sensitising potential of chemicals. *Int. J. Immunopathol. Pharmacol.* 20, 259–265.
- Boisleve, F., Kerdine-Romer, S., Rougier-Larzat, N., Pallardy, M., 2004. Nickel and DNCB induce CCR7 expression on human dendritic cells through different signalling pathways: role of TNF-alpha and MAPK. *J. Invest. Dermatol.* 123, 494–502.
- Bonneville, M., Chavagnac, C., Vocanson, M., Rozieres, A., Benetiere, J., Pernet, I., Denis, A., Nicolas, J.F., Hennino, A., 2007. Skin contact irritation conditions the development and severity of allergic contact dermatitis. *J. Invest. Dermatol.* 127, 1430–1435.
- Caux, C., Vanbervliet, B., Massacrier, C., Dezutter-Dambuyant, C., Saint-Vis, B., Jacquet, C., Yoneda, K., Imamura, S., Schmitt, D., Banchereau, J., 1996. CD34+ hematopoietic

- progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GM-CSF+TNF alpha. *J. Exp. Med.* 184, 695–706.
- Caux, C., Massacrier, C., Dubois, B., Valladeau, J., Dezutter-Dambuyant, C., Durand, I., Schmitt, D., Saeland, S., 1999. Respective involvement of TGF-beta and IL-4 in the development of Langerhans cells and non-Langerhans dendritic cells from CD34+ progenitors. *J. Leukoc. Biol.* 66, 781–791.
- Cluzel-Tailhardat, M., Bonnet-Duquenois, M., de Queral, D.P., Vocanson, M., Kurfurst, R., Courtellemont, P., Le, V.B., Nicolas, J.F., 2007. Chemicals with weak skin sensitizing properties can be identified using low-density microarrays on immature dendritic cells. *Toxicol. Lett.* 174, 98–109.
- Coulter, E.M., Farrell, J., Mathews, K.L., Maggs, J.L., Pease, C.K., Lockley, D.J., Basketter, D.A., Park, B.K., Naisbitt, D.J., 2007. Activation of human dendritic cells by p-phenylenediamine. *J. Pharmacol. Exp. Ther.* 320, 885–892.
- Coutant, K.D., de Fraissinette, A.B., Cordier, A., Ulrich, P., 1999. Modulation of the activity of human monocyte-derived dendritic cells by chemical haptens, a metal allergen, and a staphylococcal superantigen. *Toxicol. Sci.* 52, 189–198.
- Cumberbatch, M., Dearman, R.J., Antonopoulos, C., Groves, R.W., Kimber, I., 2001. Interleukin (IL)-18 induces Langerhans cell migration by a tumour necrosis factor-alpha- and IL-1beta-dependent mechanism. *Immunology* 102, 323–330.
- Cumberbatch, M., Dearman, R.J., Griffiths, C.E., Kimber, I., 2003. Epidermal Langerhans cell migration and sensitisation to chemical allergens. *APMIS* 111, 797–804.
- De Smedt, A.C., Van Den Heuvel, R.L., Zwi, B.N., Schoeters, G.E., 2001. Modulation of phenotype, cytokine production and stimulatory function of CD34+ derived DC by NiCl(2) and SDS. *Toxicol. In Vitro* 15, 319–325.
- De Smedt, A.C., Van Den Heuvel, R.L., Van Tendeloo, V.F., Berneman, Z.N., Schoeters, G.E., Weber, E., Tuschl, H., 2002. Phenotypic alterations and IL-1beta production in CD34(+) progenitor- and monocyte-derived dendritic cells after exposure to allergens: a comparative analysis. *Arch. Dermatol. Res.* 294, 109–116.
- De Smedt, A.C., Van Den Heuvel, R.L., Van Tendeloo, V.F., Berneman, Z.N., Schoeters, G.E., 2005. Capacity of CD34+ progenitor-derived dendritic cells to distinguish between sensitizers and irritants. *Toxicol. Lett.* 156, 377–389.
- Enk, A.H., Katz, S.I., 1992. Early molecular events in the induction phase of contact sensitivity. *Proc. Natl. Acad. Sci. U. S. A.* 89, 1398–1402.
- European Commission, 1986. Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. *Official Journal of the European Communities* L358, 1–29.
- European Regulation, 2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. *Official Journal of the European Union* L396, 1–849.
- Gerberick, G.F., Robinson, M.K., Ryan, C.A., Dearman, R.J., Kimber, I., Basketter, D.A., Wright, Z., Marks, J.G., 2001. Contact allergenic potency: correlation of human and local lymph node assay data. *Am. J. Contact Dermat.* 12, 156–161.
- Gerberick, G.F., Ryan, C.A., Kern, P.S., Dearman, R.J., Kimber, I., Patlewicz, G.Y., Basketter, D.A., 2004. A chemical dataset for evaluation of alternative approaches to skin-sensitization testing. *Contact Dermatitis* 50 (5), 274–288.
- Gerberick, G.F., Ryan, C.A., Kern, P.S., Schlatter, H., Dearman, R.J., Kimber, I., Patlewicz, G.Y., Basketter, D.A., 2005. Compilation of historical local lymph node data for evaluation of skin sensitization alternative methods. *Dermatitis* 16, 157–202.
- Gerberick, G.F., Vassallo, J.D., Foertsch, L.M., Price, B.B., Chaney, J.G., Lepoittevin, J.P., 2007. Quantification of chemical peptide reactivity for screening contact allergens: a classification tree model approach. *Toxicol. Sci.* 97 (2), 417–427.
- Gerberick, G.F., Aleksic, M., Basketter, D., Casati, S., Karlberg, A.T., Kern, P., Kimber, I., Lepoittevin, J.P., Natsch, A., Ovigne, J.M., Rovida, C., Sakaguchi, H., Schultz, T., 2008. Chemical reactivity measurement and the predictive identification of skin sensitizers. The report and recommendations of ECVAM Workshop 64. *Altern. Lab. Anim.* 36 (2), 215–242.
- Gildea, L.A., Ryan, C.A., Foertsch, L.M., Kennedy, J.M., Dearman, R.J., Kimber, I., Gerberick, G.F., 2006. Identification of gene expression changes induced by chemical allergens in dendritic cells: opportunities for skin sensitization testing. *J. Invest. Dermatol.* 126, 1813–1822.
- Goldberg, A.M., Hartung, T., 2006. Protecting more than animals. *Sci. Am.* 294, 84–91.
- Griem, P., Goebel, C., Scheffler, H., 2003. Proposal for a risk assessment methodology for skin sensitization based on sensitization potency data. *Regul. Toxicol. Pharmacol.* 38, 269–290.
- Herbst, B., Kohler, G., Mackensen, A., Veelken, H., Lindemann, A., 1998. GM-CSF promotes differentiation of a precursor cell of monocytes and Langerhans-type dendritic cells from CD34+ haemopoietic progenitor cells. *Br. J. Haematol.* 101, 231–241.
- Hirota, M., Moro, O., 2006. MIP-1beta, a novel biomarker for *in vitro* sensitization test using human monocyte cell line. *Toxicol. In Vitro* 20, 736–742.
- Hooyberghs, J., Schoeters, E., Lambrechts, N., Nelissen, I., Witters, H., Schoeters, G., Van Den, H.R., 2008. A cell-based *in vitro* alternative to identify skin sensitizers by gene expression. *Toxicol. Appl. Pharmacol.* 231, 103–111.
- Hulet, B.C., Rowden, G., Ryan, C.A., Lawson, C.M., Dawes, S.M., Ridder, G.M., Gerberick, G.F., 2001. Cytokine induction of a human acute myelogenous leukemia cell line (KG-1) to a CD1a+ dendritic cell phenotype. *Arch. Dermatol. Res.* 293, 147–158.
- Hulet, B.A., Ryan, C.A., Gerberick, G.F., 2002. Elucidating changes in surface marker expression of dendritic cells following chemical allergen treatment. *Toxicol. Appl. Pharmacol.* 182, 226–233.
- Hulet, B.C., Ryan, C.A., Gildea, L.A., Gerberick, G.F., 2005. Relationship of CD86 surface marker expression and cytotoxicity on dendritic cells exposed to chemical allergen. *Toxicol. Appl. Pharmacol.* 209, 159–166.
- Ikarashi, Y., Tsuchiya, T., Nakamura, A., 1992. Detection of contact sensitivity of metal salts using the murine local lymph node assay. *Toxicol. Lett.* 62, 53–61.
- Ito, T., Inaba, M., Inaba, K., Toki, J., Sogo, S., Iguchi, T., Adachi, Y., Yamaguchi, K., Amakawa, R., Valladeau, J., Saeland, S., Fukuhara, S., Ikehara, S., 1999. A CD1a+/CD11c+ subset of human blood dendritic cells is a direct precursor of Langerhans cells. *J. Immunol.* 163, 1409–1419.
- Judge, F., Boissier, C., Rougier-Larzat, N., Corlu, A., Chesne, C., Semana, G., Heresbach, D., 2005. Regulation by allergens of chemokine receptor expression on *in vitro* generated dendritic cells. *Toxicology* 212, 227–238.
- Kimber, I., Hilton, J., Weisenberger, C., 1989. The murine local lymph node assay for identification of contact allergens: a preliminary evaluation of *in situ* measurement of lymphocyte proliferation. *Contact Dermatitis* 21, 215–220.
- Kimber, I., Dearman, R.J., Basketter, D.A., Ryan, C.A., Gerberick, G.F., 2002. The local lymph node assay: past, present and future. *Contact Dermatitis* 47 (6), 315–328.
- Kimber, I., Basketter, D.A., Butler, M., Gamer, A., Garrigue, J.L., Gerberick, G.F., Newsome, C., Steiling, W., Vohr, H.W., 2003. Classification of contact allergens according to potency: proposals. *Food Chem. Toxicol.* 41, 1799–1809.
- Koeffler, H.P., Golde, D.W., 1980. Human myeloid leukemia cell lines: a review. *Blood* 56, 344–350.
- Koeffler, H.P., Golde, D.W., 1978. Acute myelogenous leukemia: a human cell line responsive to colony-stimulating activity. *Science* 200, 1153–1154.
- Koss, A., Lucero, G., Koziner, B., 1996. Granulocyte-colony stimulating factor, granulocyte-macrophage colony stimulating factor and interleukin 4 induce differentiation in the U-937 human monocytic leukemia cell line. *Leuk. Lymphoma* 22, 163–171, follow.
- Larsson, K., Lindstedt, M., Borrebaeck, C.A., 2006. Functional and transcriptional profiling of MUTZ-3, a myeloid cell line acting as a model for dendritic cells. *Immunology* 117, 156–166.
- Lee, J.S., Kim, I.S., Ryu, J.S., Yun, C.Y., 2008. House dust mite, *Dermatophagoides pteronissinus* increases expression of MCP-1, IL-6, and IL-8 in human monocyte THP-1 cells. *Cytokine* 42, 365–371.
- Levin, C.Y., Maibach, H.I., 2002. Irritant contact dermatitis: is there an immunologic component? *Int. Immunopharmacol.* 2, 183–189.
- Lim, Y.M., Moon, S.J., An, S.S., Lee, S.J., Kim, S.Y., Chang, I.S., Park, K.L., Kim, H.A., Heo, Y., 2008. Suitability of macrophage inflammatory protein-1beta production by THP-1 cells in differentiating skin sensitizers from irritant chemicals. *Contact Dermatitis* 58, 193–198.
- Manome, H., Aiba, S., Tagami, H., 1999. Simple chemicals can induce maturation and apoptosis of dendritic cells. *Immunology* 98, 481–490.
- Martin-Fontecha, A., Sebastiani, S., Hopken, U.E., Uguccioni, M., Lipp, M., Lanzavecchia, A., Sallusto, F., 2003. Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and priming. *J. Exp. Med.* 198, 615–621.
- Masterson, A.J., Sombroek, C.C., De Gruijl, T.D., Graus, Y.M., van der Vliet, H.J., Loughheed, S.M., van den Eertwegh, A.J., Pinedo, H.M., Scheper, R.J., 2002. MUTZ-3, a human cell line model for the cytokine-induced differentiation of dendritic cells from CD34+ precursors. *Blood* 100, 701–703.
- Meierhoff, G., Krause, S.W., Andreessen, R., 1998. Comparative analysis of dendritic cells derived from blood monocytes or CD34+ hematopoietic progenitor cells. *Immunobiology* 198, 501–513.
- Mitjans, M., Viviani, B., Lucchi, L., Galli, C.L., Marinovich, M., Corsini, E., 2008. Role of p38 MAPK in the selective release of IL-8 induced by chemical allergen in naive THP-1 cells. *Toxicol. In Vitro* 22, 386–395.
- Miyazawa, M., Ito, Y., Yoshida, Y., Sakaguchi, H., Suzuki, H., 2007. Phenotypic alterations and cytokine production in THP-1 cells in response to allergens. *Toxicol. In Vitro* 21, 428–437.
- Miyazawa, M., Ito, Y., Kosaka, N., Nukada, Y., Sakaguchi, H., Suzuki, H., Nishiyama, N., 2008a. Role of MAPK signaling pathway in the activation of dendritic type cell line, THP-1, induced by DNCB and NiSO4. *J. Toxicol. Sci.* 33, 51–59.
- Miyazawa, M., Ito, Y., Kosaka, N., Nukada, Y., Sakaguchi, H., Suzuki, H., Nishiyama, N., 2008b. Role of TNF-alpha and extracellular ATP in THP-1 cell activation following allergen exposure. *J. Toxicol. Sci.* 33, 71–83.
- Mizuashi, M., Ohtani, T., Nakagawa, S., Aiba, S., 2005. Redox imbalance induced by contact sensitizers triggers the maturation of dendritic cells. *J. Invest. Dermatol.* 124, 579–586.
- Moed, H., Boersma, D.M., Stof, T.J., von Blomberg, B.M., Bruynzeel, D.P., Scheper, R.J., Gibbs, S., Rustemeyer, T., 2004. Nickel-responding T cells are CD4+ CLA+ CD45RO+ and express chemokine receptors CXCR3, CCR4 and CCR10. *Br. J. Dermatol.* 151, 32–41.
- Montellius, J., Wahlkvist, H., Boman, A., Wahlberg, J.E., 1998. Murine local lymph node assay for predictive testing of allergenicity: two irritants caused significant proliferation. *Acta Derm. Venereol.* 78, 433–437.
- Nukada, Y., Miyazawa, M., Kosaka, N., Ito, Y., Sakaguchi, H., Nishiyama, N., 2008. Production of IL-8 in THP-1 cells following contact allergen stimulation via mitogen-activated protein kinase activation or tumor necrosis factor-alpha production. *J. Toxicol. Sci.* 33, 175–185.
- OECD, 2005. OECD Series on Testing and Assessment No 34. Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment. Paris, France Organisation for Economic Cooperation and Development 96pp.
- Ouweland, K., Santegoets, S.J., Bruynzeel, D.P., Scheper, R.J., De Gruijl, T.D., Gibbs, S., 2008. CXCL12 is essential for migration of activated Langerhans cells from epidermis to dermis. *Eur. J. Immunol.* 38, 3050–3059.

- Ozawa, H., Nakagawa, S., Tagami, H., Aiba, S., 1996. Interleukin-1 beta and granulocyte-macrophage colony-stimulating factor mediate Langerhans cell maturation differently. *J. Invest. Dermatol.* 106, 441–445.
- Pichowski, J.S., Cumberbatch, M., Dearman, R.J., Basketter, D.A., Kimber, I., 2000. Investigation of induced changes in interleukin 1beta mRNA expression by cultured human dendritic cells as an *in vitro* approach to skin sensitization testing. *Toxicol. In Vitro* 14, 351–360.
- Pichowski, J.S., Cumberbatch, M., Dearman, R.J., Basketter, D.A., Kimber, I., 2001. Allergen-induced changes in interleukin 1 beta (IL-1 beta) mRNA expression by human blood-derived dendritic cells: inter-individual differences and relevance for sensitization testing. *J. Appl. Toxicol.* 21, 115–121.
- Ponec, M., Gibbs, S., Pilgram, G., Boelsma, E., Koerten, H., Bouwstra, J., Mommaas, M., 2001. Barrier function in reconstructed epidermis and its resemblance to native human skin. *Skin Pharmacol. Appl. Skin Physiol.* 14 (Suppl. 1), 63–71.
- Python, F., Goebel, C., Aeby, P., 2007. Assessment of the U937 cell line for the detection of contact allergens. *Toxicol. Appl. Pharmacol.* 220, 113–124.
- Rambukkana, A., Bos, J.D., Irik, D., Menko, W.J., Kapsenberg, M.L., Das, P.K., 1995. In situ behavior of human Langerhans cells in skin organ culture. *Lab. Invest.* 73, 521–531.
- Regnier, M., Staquet, M.J., Schmitt, D., Schmidt, R., 1997. Integration of Langerhans cells into a pigmented reconstructed human epidermis. *J. Invest. Dermatol.* 109, 510–512.
- Roberts, D.W., Aptula, A.O., Patlewicz, G., 2007a. Electrophilic chemistry related to skin sensitization. Reaction mechanistic applicability domain classification for a published data set of 106 chemicals tested in the mouse local lymph node assay. *Chem. Res. Toxicol.* 20, 44–60.
- Roberts, D.W., Patlewicz, G., Kern, P.S., Gerberick, F., Kimber, I., Dearman, R.J., Ryan, C.A., Basketter, D.A., Aptula, A.O., 2007b. Mechanistic applicability domain classification of a local lymph node assay dataset for skin sensitization. *Chem. Res. Toxicol.* 20, 1019–1030.
- Rougier, N., Redziniak, G., Mouglin, D., Schmitt, D., Vincent, C., 2000. *In vitro* evaluation of the sensitization potential of weak contact allergens using Langerhans-like dendritic cells and autologous T cells. *Toxicology* 145, 73–82.
- Rustemeyer, T., Preuss, M., von Blomberg, B.M., Das, P.K., Scheper, R.J., 2003. Comparison of two *in vitro* dendritic cell maturation models for screening contact sensitizers using a panel of methacrylates. *Exp. Dermatol.* 12, 682–691.
- Rustemeyer, T., von Blomberg, B.M., van Hoogstraten, I.M., Bruynzeel, D.P., Scheper, R.J., 2004. Analysis of effector and regulatory immune reactivity to nickel. *Clin. Exp. Allergy* 34, 1458–1466.
- Ryan, C.A., Cruse, L.W., Skinner, R.A., Dearman, R.J., Kimber, I., Gerberick, G.F., 2002. Examination of a vehicle for use with water soluble materials in the murine local lymph node assay. *Food Chem. Toxicol.* 40, 1719–1725.
- Ryan, C.A., Gildea, L.A., Huilette, B.C., Dearman, R.J., Kimber, I., Gerberick, G.F., 2004. Gene expression changes in peripheral blood-derived dendritic cells following exposure to a contact allergen. *Toxicol. Lett.* 150, 301–316.
- Sakaguchi, H., Ashikaga, T., Miyazawa, M., Yoshida, Y., Ito, Y., Yoneyama, K., Hirota, M., Itagaki, H., Toyoda, H., Suzuki, H., 2006. Development of an *in vitro* skin sensitization test using human cell lines; human Cell Line Activation Test (h-CLAT). II. An inter-laboratory study of the h-CLAT. *Toxicol. In Vitro* 20, 774–784.
- Sakaguchi, H., Ashikaga, T., Miyazawa, M., Kosaka, N., Ito, Y., Yoneyama, K., Sono, S., Itagaki, H., Toyoda, H., Suzuki, H., 2008. The relationship between CD86/CD54 expression and THP-1 cell viability in an *in vitro* skin sensitization test – human cell line activation test (h-CLAT). *Cell Biol. Toxicol.*
- Sakaguchi, H., Miyazawa, M., Yoshida, Y., Ito, Y., Suzuki, H., 2007. Prediction of preservative sensitization potential using surface marker CD86 and/or CD54 expression on human cell line, THP-1. *Arch. Dermatol. Res.* 298, 427–437.
- Sallusto, F., Lanzavecchia, A., 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J. Exp. Med.* 179, 1109–1118.
- Santegoets, S.J., Schreurs, M.W., Masterson, A.J., Liu, Y.P., Goletz, S., Baumeister, H., Kueter, E.W., Loughheed, S.M., van den Eertwegh, A.J., Scheper, R.J., Hooijberg, E., De Gruijl, T.D., 2006. *In vitro* priming of tumor-specific cytotoxic T lymphocytes using allogeneic dendritic cells derived from the human MUTZ-3 cell line. *Cancer Immunol. Immunother.* 55, 1480–1490.
- Santegoets, S.J., van den Eertwegh, A.J., van de Loosdrecht, A.A., Scheper, R.J., De Gruijl, T.D., 2008. Human dendritic cell line models for DC differentiation and clinical DC vaccination studies. *J. Leukoc. Biol.* 84 (6), 1364–1373.
- Schoeters, E., Verheyen, G.R., Van Den, H.R., Nelissen, I., Witters, H., Van Tendeloo, V., Schoeters, G.E., Berneman, Z.N., 2005. Expression analysis of immune-related genes in CD34(+) progenitor-derived dendritic cells after exposure to the chemical contact allergen DNCB. *Toxicol. In Vitro* 19, 909–913.
- Schoeters, E., Nuijten, J.M., Van Den Heuvel, R.L., Nelissen, I., Witters, H., Schoeters, G.E., Van Tendeloo, V., Berneman, Z.N., Verheyen, G.R., 2006. Gene expression signatures in CD34+ progenitor-derived dendritic cells exposed to the chemical contact allergen nickel sulfate. *Toxicol. Appl. Pharmacol.* 216, 131–149.
- Schoeters, E., Verheyen, G.R., Nelissen, I., Van Rompay, A.R., Hooyberghs, J., Van Den Heuvel, R.L., Witters, H., Schoeters, G.E., Van Tendeloo, V., Berneman, Z.N., 2007. Microarray analyses in dendritic cells reveal potential biomarkers for chemical-induced skin sensitization. *Mol. Immunol.* 44, 3222–3233.
- Selgrade, M., Boykin, E.H., Haykal-Coates, N., Woolhiser, M.R., Wiescinski, C., Andrews, D.L., Farraj, A.K., Doerfler, D.L., Gavett, S.H., 2006. Inconsistencies between cytokine profiles, antibody responses, and respiratory hyperresponsiveness following dermal exposure to isocyanates. *Toxicol. Sci.* 94, 108–117.
- Smith, C.K., Hotchkiss, S.A.M., 2001. Allergic Contact Dermatitis: Chemical and Metabolic Mechanisms. 1st ed. London Taylor and Francis.
- Smith, H.R., Basketter, D.A., McFadden, J.P., 2002. Irritant dermatitis, irritancy and its role in allergic contact dermatitis. *Clin. Exp. Dermatol.* 27, 138–146.
- Staquet, M.J., Sportouch, M., Jacquet, C., Schmitt, D., Guesnet, J., Peguet-Navarro, J., 2004. Moderate skin sensitizers can induce phenotypic changes on *in vitro* generated dendritic cells. *Toxicol. In Vitro* 18, 493–500.
- Straube, F., Grenet, O., Bruegger, P., Ulrich, P., 2005. Contact allergens and irritants show discrete differences in the activation of human monocyte-derived dendritic cells: consequences for *in vitro* detection of contact allergens. *Arch. Toxicol.* 79, 37–46.
- Sundstrom, C., Nilsson, K., 1976. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). *Int. J. Cancer* 17, 565–577.
- Szameit, S., Vierlinger, K., Farmer, L., Tuschl, H., Noehammer, C., 2008. Microarray-based *in vitro* test system for the discrimination of contact allergens and irritants: identification of potential marker genes. *Clin. Chem.* 54, 525–533.
- Teobald, I., Dunnion, D.J., Whitbread, M., Curnow, S.J., Browning, M.J., 2008. Phenotypic and functional differentiation of KG-1 into dendritic-like cells. *Immunobiology* 213, 75–86.
- Tietze, C., Blomeke, B., 2008. Sensitization assays: monocyte-derived dendritic cells versus a monocyte cell line (THP-1). *J. Toxicol. Environ. Health A* 71, 965–968.
- Toebak, M.J., Moed, H., von Blomberg, M.B., Bruynzeel, D.P., Gibbs, S., Scheper, R.J., Rustemeyer, T., 2006a. Intrinsic characteristics of contact and respiratory allergens influence production of polarizing cytokines by dendritic cells. *Contact Dermatitis* 55, 238–245.
- Toebak, M.J., Pohlmann, P.R., Sampat-Sardjoepersad, S.C., von Blomberg, B.M., Bruynzeel, D.P., Scheper, R.J., Rustemeyer, T., Gibbs, S., 2006b. CXCL8 secretion by dendritic cells predicts contact allergens from irritants. *Toxicol. In Vitro* 20, 117–124.
- Toebak, M.J., de Rooij, J., Moed, H., Stoof, T.J., von Blomberg, B.M., Bruynzeel, D.P., Scheper, R.J., Gibbs, S., Rustemeyer, T., 2008. Differential suppression of dendritic cell cytokine production by anti-inflammatory drugs. *Br. J. Dermatol.* 158, 225–233.
- Tsuchiya, S., Yamabe, M., Yamaguchi, Y., Kobayashi, Y., Konno, T., Tada, K., 1980. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). *Int. J. Cancer* 26, 171–176.
- Tsuchiya, S., Kobayashi, Y., Goto, Y., Okumura, H., Nakae, S., Konno, T., Tada, K., 1982. Induction of maturation in cultured human monocytic leukemia cells by a phorbol diester. *Cancer Res.* 42, 1530–1536.
- Tuschl, H., Kovac, R., 2001. Langerhans cells and immature dendritic cells as model systems for screening of skin sensitizers. *Toxicol. In Vitro* 15, 327–331.
- Verheyen, G.R., Schoeters, E., Nuijten, J.M., Van Den Heuvel, R.L., Nelissen, I., Witters, H., Van Tendeloo, V.F., Berneman, Z.N., Schoeters, G.E., 2005. Cytokine transcript profiling in CD34+ progenitor derived dendritic cells exposed to contact allergens and irritants. *Toxicol. Lett.* 155, 187–194.
- Weltzien, H.U., Moulon, C., Martin, S., Padovan, E., Hartmann, U., Kohler, J., 1996. T cell immune responses to haptens. Structural models for allergic and autoimmune reactions. *Toxicology* 107, 141–151.
- Yoshida, Y., Sakaguchi, H., Ito, Y., Okuda, M., Suzuki, H., 2003. Evaluation of the skin sensitization potential of chemicals using expression of co-stimulatory molecules, CD54 and CD86, on the naive THP-1 cell line. *Toxicol. In Vitro* 17, 221–228.

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