

Reactivity of Langerhans cells in human reconstructed epidermis to known allergens and UV radiation

V. Facy *, V. Flouret, M. Régnier, R. Schmidt

L'OREAL Recherche, Centre Charles Zviak, 90 rue du Général Roguet, 92583 Clichy Cedex, France

Received 11 October 2004; accepted 17 March 2005

Available online 25 May 2005

Abstract

Epidermal Langerhans cells are the outmost guards of our immune defence system. These cells are directly involved in phenomena such as contact hypersensitivity and UV-induced immunosuppression. Some years ago we succeeded in introducing CD34⁺-derived Langerhans cells into a reconstructed human epidermis. Here we describe their reactivity after topical exposure of the reconstructed epidermis to known allergens, allergen-inducible cytokines, irritants and UV irradiation. Exposure to allergens for 24 h resulted in an activated appearance of the Langerhans cells and in some cases a decrease in their number. Concomitantly, IL-1 β and CD86 mRNA over-expressions were detected in the reconstructed epidermis. A topical treatment with TNF- α or IL-1 β revealed that both cytokines induced an activated appearance of the Langerhans cells as early as 4 h following application. Irritants had no effect on the integrated Langerhans cells. Exposure of the reconstructed epidermis to Solar Simulated Radiation caused a dramatic decrease in the number of Langerhans cells and a loss of dendricity in the remaining cells 24 h after irradiation. The topical application of a large spectrum UVA/B filter before irradiation prevented these UV-induced alterations. In our hands, this model provides a promising tool to evaluate the sensitization potential of new compounds and to validate the efficacy of sunscreens to prevent UV-induced immunosuppression.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: Contact hypersensitivity; Allergens; Immunosuppression; In vitro screening; Skin

1. Introduction

Langerhans cells are the immunocompetent cells of the epidermis and play a key role in contact hypersensitivity (Kimber et al., 1998, 2002; Krasteva et al., 1999). These dendritic cells capture low molecular-weight aller-

gens bound to epidermal proteins which induces their activation and migration from the epidermis to the regional lymph nodes where they present the processed antigen to naive T cells (sensitization phase). Epidermal allergen-inducible cytokines such as TNF- α and IL-1 β are involved in the Langerhans cell migration. The sensitization phase is characterized by morphological, phenotypic and functional modifications of the Langerhans cells. Co-stimulatory molecules such as CD86 appear at the surface of the Langerhans cells and participate in T cell stimulation. The elicitation phase is initiated when Langerhans cells are exposed for a subsequent time to the same antigen, triggering the activation of memory T cells which causes the recruitment of infiltrating cells into skin and as a result the allergic inflammation.

Abbreviations: AEC, 3-amino-ethyl-carbazole; DNFB, dinitrofluorobenzene; GM-CSF, granulocyte macrophage-colony stimulating factor; HES, hemalun eosin saffron; HPRT, hypoxanthine-guanine phosphoribosyltransferase; pPD, p-phenylenediamine; SCF, stem cell factor; SLS, sodium lauryl sulfate; SSR, solar simulated radiation; TGF- β , tumor growth factor- β ; TNF- α , tumor necrosis factor- α .

* Corresponding author. Tel.: +33 1 47 56 76 70; fax: +33 1 47 56 78 36.

E-mail address: vfacy@rd.loreale.com (V. Facy).

UV exposure of the skin has been shown to cause a phenomenon generally described as immunosuppression (Kripke and Morison, 1985; Ullrich, 1995; Kelly et al., 1998). This type of immunosuppression is characterized by a reduction in the number of the epidermal Langerhans cells, an alteration of their morphology and a depletion of certain cell surface markers (Aberer et al., 1981; Bacci et al., 1998; Meunier, 1999).

To assess the sensitizing potential of new chemicals or formulations, only a few *in vitro* methods exist (reviewed in Ryan et al., 2001). Important progress was made by successfully integrating CD34⁺-derived Langerhans cells into reconstructed human epidermis (Régnier et al., 1997) which do not spontaneously emigrate from epidermis as observed in skin organ cultures (Larsen et al., 1990).

In this paper, we used Langerhans cells integrated into a human epidermis reconstructed on a collagenic support (Tinois et al., 1991, 1994) to evaluate their reactivity (cell morphology, density and IL-1 β /CD86 mRNA expressions) to topically applied allergens and allergen-inducible cytokines. We also evaluated the immunophoto-protective potential of a sunscreen and compared the generated data to those obtained *in vivo* (Elmets et al., 1992; Neale et al., 1997; Ullrich et al., 1999; Moyal and Fournanier, 2001).

2. Materials and methods

2.1. Cells and culture conditions

Normal human keratinocytes extracted from mammary skin (plastic surgery), were grown in submerged cultures (Rheinwald and Green, 1975) and used at their third passage. Keratinocytes from the same donor were used to reconstruct epidermis.

CD34⁺ hematopoietic progenitors purified from cord blood cells by MACS-immunomagnetic sorting (Miltenyi Biotec, Auburn, CA) were supplied by Dr. Hatzfeld (C.N.R.S. Villejuif, France). The CD34⁺ cells were grown and differentiated into a dendritic sub-population for 7 days in StemBio A medium (C.N.R.S. Villejuif, France) supplemented with 2000 U/ml GM-CSF (Leucomax[®] 150, kindly provided by Novartis Pharma, Rueil Malmaison, France), 20 U/ml TNF- α (210-TA-010, R&D Systems, Abingdon, UK), 20 ng/ml SCF (255-SC, R&D Systems) (Caux et al., 1992). SCF was replaced at day 4 by 0.5 ng/ml TGF- β (100-B, R&D Systems) (Strobl et al., 1996).

2.2. Reconstruction of the human epidermis containing Langerhans cells

CD34⁺-derived dendritic cells (5×10^4 CD1a⁺) and 4×10^5 heterologous keratinocytes were co-seeded onto the Episkin[®] support (1.1 cm²). Cells were submerged

for 3 days in Dulbecco's modified Eagle medium/Ham F12 (3:1), containing 10% fetal calf serum, 10 ng/ml epidermal growth factor, 400 ng/ml hydrocortisone, 10^{-6} M isoproterenol, 5 μ g/ml transferrin, 2×10^{-9} M triiodothyronine, 1.8×10^{-4} M adenine and 5 μ g/ml insulin. The cells were then put for 10 days at the air–liquid interface (to induce the epidermal differentiation) in culture medium without isoproterenol, transferrin, triiodothyronine and adenine.

2.3. Immunohistochemical analysis

Hemalun Eosin Saffron (HES) staining was performed on epidermal formol-fixed sections.

Indirect immunostaining on epidermal cryosections (5 μ m) or sheets (peeled with 2 M sodium bromide and fixed with acetone) was performed using a streptavidin-biotin peroxidase assay (LSAB[®] 2 System HRP, DAKO, Glostrup, Denmark). To detect Langerhans cells in the reconstructed epidermis, the monoclonal antibodies CD1a (clone SK9, Becton Dickinson, San Jose, CA) and Langerin (clone DCGM4, Beckman Coulter/Immunotech, Marseille, France) were used at 1:30 and 1:300 dilutions, respectively. Cell nuclei were counterstained with hematoxylin in the cryosections.

2.4. Chemical treatment

Reconstructed epidermis was treated with topically applied cytokines, allergens or irritants (4 μ l spread on the 1.1 cm² area using a curved Pasteur pipette).

TNF- α and IL-1 β were purchased from R&D Systems (Abingdon, UK). Contact allergens: dinitrofluorobenzene (DNFB), oxazolone, *p*-phenylenediamine (pPD), NiSO₄, eugenol, benzocaine and irritants: sodium lauryl sulfate (SLS) and benzalkonium chloride were purchased from SIGMA (St Louis, MO) except eugenol from ACROS ORGANICS (New Jersey, USA). All allergens (except NiSO₄) were solubilized in acetone/olive oil 4:1, while irritants and NiSO₄ were dissolved in distilled water. Controls were performed with acetone/olive oil and water, respectively. The chemicals were used at a ten-fold dilution of their minimal toxic dose. The toxic dose induced decreasing dendricity associated with positivity to active caspase-3 antibody for Langerhans cells.

2.5. RT-PCR analysis

Epidermal sheets were peeled from the support using forceps or separated by dispase treatment if necessary (2.4 U/ml, Boehringer Mannheim, Germany). Total RNA was extracted with the Trizol[®] reagent (Life Technologies/GibcoBRL, Cergy Pontoise, France). First strand cDNA was synthesized from 1 μ g RNA using an oligo(dT)₁₈ primer (First-strand cDNA synthesis kit, Amersham Pharmacia Biotech, Buckinghamshire,

England). cDNA (corresponding to 0.1 µg RNA) was amplified in a total volume of 100 µl containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, 200 µM dNTPs, specific PCR primers (200 pmol IL-1β and CD86 or 5 pmol hypoxanthine-guanine phosphoribosyltransferase (HPRT)) and 2.5 units of AmpliTaq[®] DNA polymerase (Perkin Elmer). The mixture underwent 40 PCR cycles (Gene Amp[®] PCR system 2400, Perkin Elmer) with denaturation at 94 °C for 45 s, annealing at 60 °C (IL-1β/HPRT) or 55 °C (CD86) for 1 min and polymerization at 72 °C for 2 min. The following primers (Genset, Paris, France) were used (the 5' primer was 6-Fam labeled):

IL-1β (Reutter et al., 1997; 250 pb PCR product): 5'-(6-Fam)GAC ACA TGG GAT AAC GAG GC 5'-ACG CAG GAC AGG TAC AGA TT

CD86 (Jellis et al., 1995; 212 pb PCR product): 5'-(6-Fam)CAC GGT TAC CCA GAA CCT AAG 5'-GGT GAA GAT AAA AGC CGC GTC

HPRT (housekeeping gene; 470 pb PCR product): 5'-(6-Fam)GGC GTC GTG ATT AGT GAT GAT GAT GAA CC 5'-CTT GCG ACC TTG ACC ATC TTT GGA

The PCR products were visualized on a 2% agarose/Tris-acetate EDTA gel containing ethidium bromide. The excess of primers was detectable by the Fam fluorescent labeling. The PCR products were quantified by fluorometric detection after capillary electrophoretic separation using ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The IL-1β/HPRT and CD86/HPRT fluorometric intensities in the allergen-treated samples were compared to the untreated control.

2.6. UV source and irradiation procedure

Solar simulated radiations (SSR) were generated from a 1000 W Xenon lamp (Oriel) filtered with a WG 320, 1.5 mm filter (Schott).

Wavelength spectra control and irradiance measurements were performed using a spectroradiometer (Macam).

Mexoryl[®] SX (terephthalylidene dicamphor sulfonic acid), a UVB/UVA filter diluted in an oil/water emulsion was applied topically (2 mg/cm²) onto the reconstructed epidermis before a 8.4 J/cm² SSR exposure; 8.4 J/cm² SSR was the minimal toxic dose inducing sunburn cells.

3. Results

3.1. Immunohistochemical analysis of reconstructed epidermis containing Langerhans cells

Histological analysis of the reconstructed epidermis showed a normal stratified epithelium (Fig. 1a). The

Langerin staining of cryosections detected the integrated Langerhans cells mainly in the suprabasal epidermal layers (Fig. 1b). A homogeneous distribution of the dendritic Langerhans cells was observed in the epidermal sheet (Fig. 1c).

3.2. Reactivity of the Langerhans cells to allergens and allergen-inducible cytokines

3.2.1. Cell morphology

To investigate the effect of topically applied allergens on Langerhans cell morphology, we performed Langerin immunostaining in the epidermal sheets 24 h after treatment. DNFB (0.2%), oxazolone (1%), pPD (1%), NiSO₄ (5%), eugenol (2.5%) and benzocaine (5%) induced in 88% of experiments an activated appearance of the Langerhans cells, characterized by a higher staining in the cellular body and shorter dendrites, accompanied in 20–25% of assays by a decrease in the number of Langerhans cells. The irritants SLS (1%) and benzalkonium chloride (0.1%) and allergens used at a tenfold lower dose or applied for less than 21 h, had no effect on cell morphology (Fig. 2).

To test the reactivity of the integrated Langerhans cells to known allergen-inducible cytokines, we analyzed their morphology by CD1a immunostaining in the epidermal sheet. Topically applied TNF-α (100 U/ml) or IL-1β (500 U/ml) for 2, 4, 6 or 24 h showed that both cytokines induced an activated appearance of the Langerhans cells as early as 4 h after treatment, characterized by a higher staining in the cellular body and shorter dendrites. The changes were more pronounced at 24 h (Fig. 3).

3.2.2. IL-1β and CD86 mRNA expressions

IL-1β and CD86 mRNA expressions in reconstructed epidermis containing Langerhans cells were analysed to test the reactivity of Langerhans cells to allergens.

A 24 h exposure of the reconstructed epidermis to the topically applied allergens DNFB (0.2%), oxazolone (1%), pPD (1%), NiSO₄ (5%), eugenol (2.5%) and benzocaine (5%) resulted in IL-1β and CD86 mRNA over-expressions, whereas the irritants SLS (1%) and benzalkonium chloride (0.1%) had no effect (Fig. 4).

A comparative study with Langerhans cell-free epidermis showed an over-expression of IL-1β mRNA after a 24 h exposure to both DNFB and oxazolone, but not to pPD and eugenol (Fig. 5). A constitutive IL-1β mRNA expression was observed in the untreated Langerhans cell-free reconstructed epidermis. Exposure to the above allergens did not induce CD86 mRNA expression which was not constitutively present in the absence of Langerhans cells (data not shown).

3.3. Inter-donor variation

27 “donors” of CD34⁺ were analysed (17 donors alone and 10 pairs of donors). Langerhans cell reactivity

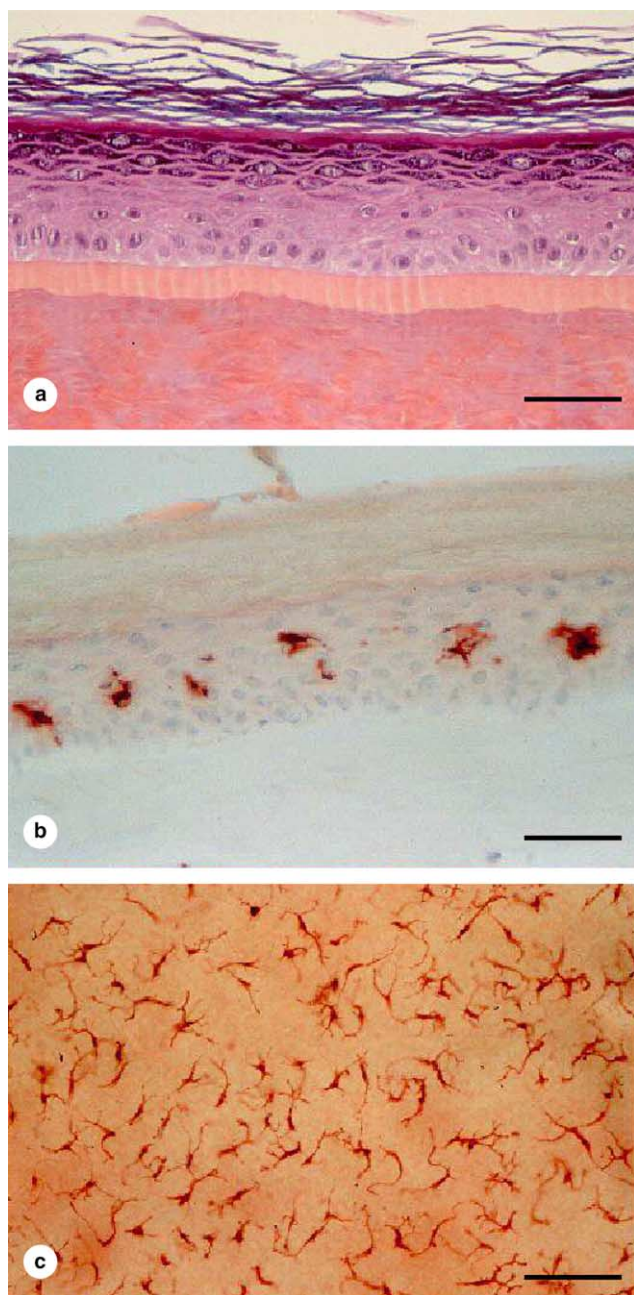


Fig. 1. Immunohistochemical analysis of reconstructed epidermis containing Langerhans cells. Histochemical analysis of reconstructed epidermis containing Langerhans cells was performed using HES staining (a), anti-langerin staining (peroxidase/AEC reaction) with hematoxylin counterstaining was performed on cryosection to localize Langerhans cells inner the epidermal layers (b; the brown cells are the Langerin-positive cells) and Langerin immunostaining (c) on epidermal sheets visualized the dense network of the dendritic Langerhans cells. Scale bars: 50 μ m (a and b), 100 μ m (c).

showed a CD34⁺ cell donor variability (Table 1). The allergen-induced morphology variation was observed for 88% of donors (between 76% and 93% depending on the chemical). The allergen-induced mRNA IL-1 β over-expression was observed for 58% of donors

(between 50% and 77%) and mRNA CD86 over-expression for 58% of donors (between 33% and 67%). In most cases, 1 donor (or pair of donors) responded to all allergens or did not respond. CD86 mRNA over-expression was observed in almost all IL-1 β -positive responders. The IL-1 β and CD86 over-expressions varied from 1.1 to 8.0 times compared to the untreated samples, depending on the allergen and the CD34⁺ cell donor.

Data showed that allergens and irritants can be discriminated.

3.4. UV-induced damage and protection of Langerhans cells

Exposure of the reconstructed epidermis to 8.4 J/cm² SSR caused a dramatic decrease in the number of Langerin-positive cells and loss of dendricity in the remaining cells 24 h after irradiation. Topical application of a UVA/B filter (Mexoryl[®] SX at 2% or more) before irradiation completely prevented these UV-induced alterations (Fig. 6).

4. Discussion

Using the collagenic Episkin[®] dermal support, the reconstructed epidermis containing CD34⁺-derived Langerhans cells exhibits a normal epidermal stratification with Langerhans cells in the suprabasal cell layers. Their density (30000 cells/cm²) is comparable to that in normal human skin (Schempp et al., 2000). Advantageously compared to skin organ cultures where Langerhans cells emigrate spontaneously from the epidermis, Langerhans cells in the reconstructed epidermis remain resident cells. Immunohistochemical and electron microscopic analysis revealed that the integrated Langerhans cells exhibited the main characteristic features of epidermal Langerhans cells such as Birbeck granules and CD1a surface antigens (Régnier et al., 1997). The present investigation was performed to pre-validate this model for its use as an alternative to animal experiments by studying the reactivity of the integrated Langerhans cells to allergens, allergen-inducible cytokines, and to UV exposure.

We investigated the effect of topically applied allergens on the Langerin-positive cell morphology in reconstructed epidermis. DNFB, oxazolone, PPD, NiSO₄, eugenol and benzocaine led Langerhans cells to acquire an activated appearance at 24 h, characterized by a higher staining in the cellular body and shorter dendrites, sometimes accompanied by a decrease in the number of Langerhans cells. These changes were dependent on the exposure time and the dose of the allergen. Interestingly, the active allergen concentrations in our epidermal model were very close to the doses used in patch tests (de Groot et al., 1994). Irritants had no significant effect on Langerhans cell morphology.

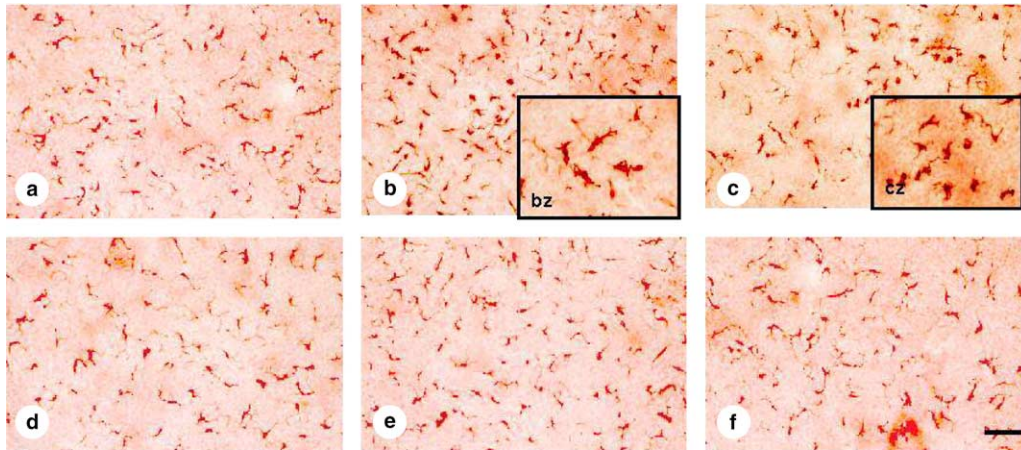


Fig. 2. Dose- and time-dependent effect of a topically applied allergen on the Langerin-positive cells in reconstructed epidermis. Immunostaining was performed in the epidermal sheet treated with solvent (a; acetone/oil shown, water was similar), DNFB 0.2% for 24 h (b; bz: zoom), 48 h (c; cz: zoom), for 3, 5, 18, or 21 h (d), DNFB 0.02% (e) and SLS 1% (f). Scale bar: 100 μ m (a–f).

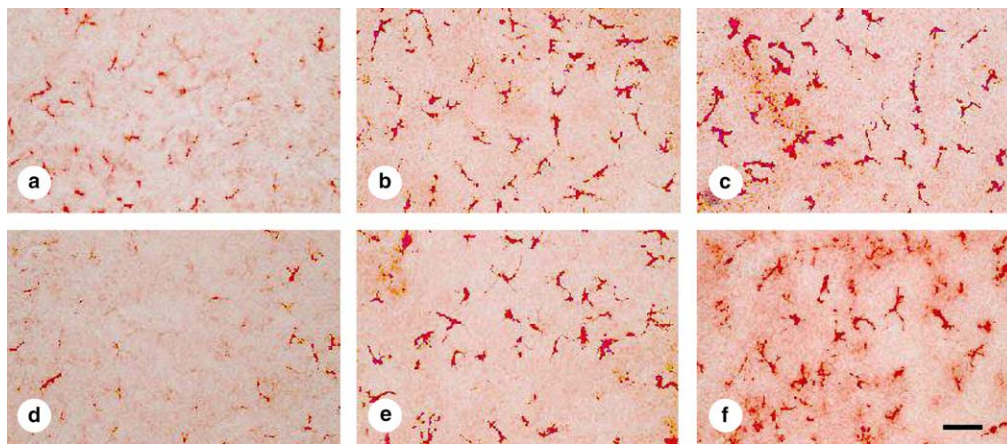


Fig. 3. Time-dependent effect of topically applied allergen-inducible cytokines on the CD1a-positive cells in reconstructed epidermis. Immunostaining was performed in the epidermal sheet after IL-1 β (500 U/ml; a, b and c) or TNF- α (100 U/ml; d, e and f) treatment for 0–2 h (a, d); 4–6 h (b, e) and 24 h (c, f). Scale bar: 100 μ m (a–f).

It has been previously demonstrated that TNF- α and IL-1 β induced changes in Langerhans cell morphology and that these cytokines were involved in the initiation of cell migration after contact sensitization in mice and in humans (Cumberbatch et al., 1997, 1999). Our results showed that both TNF- α and IL-1 β induced an activated appearance of the integrated Langerhans cells as early as 4 h after exposure, characterized by a more compact dendritic morphology accompanied by an increase in cell size, as already described *in vivo* in murine epidermis (Cumberbatch et al., 1997).

The observed changes were observed with both CD1a (Fithian et al., 1981) and Langerin (Valladeau et al., 1999, 2000) markers. Since Langerin has been shown to be still expressed in dendritic cells after allergen-induced migration from the epidermis to lymph nodes in mouse (Stoitzner et al., 2003), the observed reduction of the Langerin-positive cells in the model most likely reflected an emigration of Langerhans cells rather than a loss of

the detected marker. Unlike in epidermis reconstructed on dead de-epidermized dermis (Régner et al., 1997), cell migration from the epidermis reconstructed on the Epi-skin® dermal support is more difficult to evidence due to the dense and probably cell-impermeable lamination of the collagen IV over collagen I of the support.

It was reported that exposure of a human skin organ culture to contact allergens resulted in a transient IL-1 β mRNA over-expression some hours later (Matsunaga et al., 1998). In mouse, the IL-1 β mRNA over-expression has been shown to be induced as early as 15 min after skin painting with allergens and lasting for 24 h (Enk and Katz, 1992). Based on these observations, we have examined the effect of allergens on the IL-1 β mRNA expression in the reconstructed epidermis with and without Langerhans cells. All applied allergens induced an IL-1 β mRNA over-expression after 24 h exposure. This over-expression was not detectable after 5 h (data not shown). Irritants had no effect. A comparative

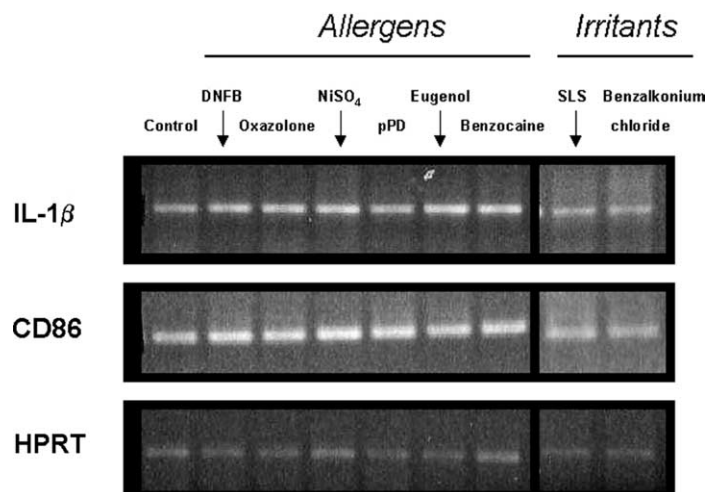


Fig. 4. IL-1 β and CD86 mRNA expressions in allergen-treated reconstructed epidermis containing Langerhans cells. RT-PCR was performed after exposure of epidermis for 24 h to solvent (acetone/oil shown, water was similar), DNFB (0.2%), oxazolone (1%), NiSO₄ (5%), pPD (1%), eugenol (2.5%), benzocaine (5%), SLS (1%) and benzalkonium chloride (0.1%). IL-1 β , CD86 and HPRT (housekeeping gene) PCR products were detected after electrophoresis in the presence of ethidium bromide.

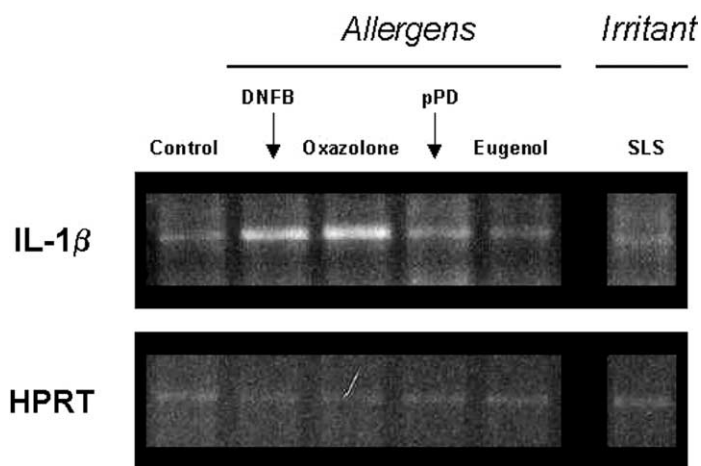


Fig. 5. IL-1 β mRNA expression in allergen-treated reconstructed epidermis without Langerhans cells. RT-PCR was performed after exposure of epidermis for 24 h to solvent (acetone/oil shown, water was similar), DNFB (0.2%), oxazolone (1%), pPD (1%), eugenol (2.5%) and SLS (1%). IL-1 β and HPRT (housekeeping gene) PCR products were detected after electrophoresis in the presence of ethidium bromide.

Table 1
Ratio and percentage (values in parentheses) of positive^a/total experiments

Reactivity	Allergens						Irritants	
	DNFB	Oxazolone	pPD	NiSO ₄	Eugenol	Benzocaine	SLS	Benzalkonium chloride
Dendrite retraction	18/20 (90)	13/14 (93)	15/17 (88)	9/10 (90)	13/17 (76)	8/9 (89)	2/9 (22)	0/2 (0)
IL-1 β mRNA over-expression	10/13 (77)	4/7 (57)	6/11 (55)	2/4 (50)	4/8 (50)	0/1 (0)	1/6 (17)	0/2 (0)
CD86 mRNA over-expression	8/12 (67)	3/5 (60)	6/8 (75)	1/3 (33)	4/7 (57)	–	1/6 (17)	0/2 (0)

^a Which induced Langerhans cell reactivity.

study in reconstructed epidermis without Langerhans cells revealed that only strong allergens like DNFB and oxazolone induced an increase of IL-1 β mRNA expression, whereas the weaker allergens pPD and eugenol as well as the irritant SLS had no effect. It has been

already shown that keratinocytes were the main source of IL-1 β release, 24 h after a DNFB treatment of skin organ cultures (Rambukkana et al., 1996).

Based on numerous reports showing that dendritic cells increase CD86 cell surface antigen expression after

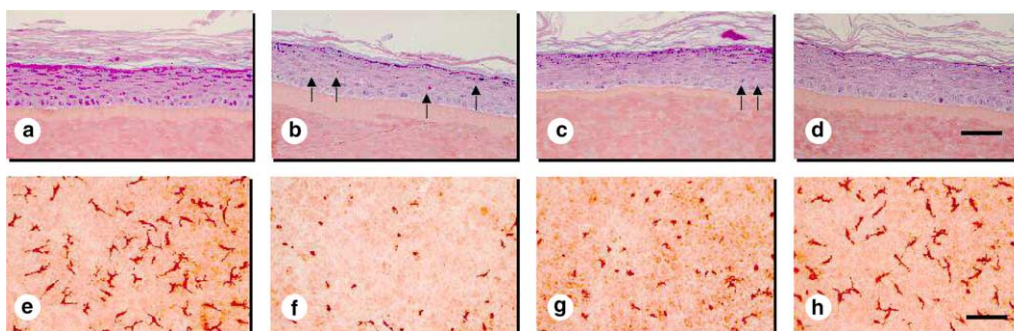


Fig. 6. Effect of SSR exposure on reconstructed epidermis containing Langerhans cells. Protection by a UVA/B sunscreen. Histological sections (a, b, c and d) and Langerin immunostaining of the respective epidermal sheets (e, f, g and h) were performed 24 h after a 8.4 J/cm² SSR. No irradiated control (a, e); SSR + vehicle (b, f); SSR + 1% Mexoryl[®] SX (c, g) and SSR + 4% Mexoryl[®] SX (d, h). Sunburn cells are indicated by →. Scale bars: 100 μ m (a–h).

allergen exposure (Aiba et al., 1997, 2000; Manome et al., 1999; Coutant et al., 1999; Rougier et al., 2000; Rousset et al., 2000; Tuschl and Kovac, 2001), we have analyzed CD86 mRNA expression in the reconstructed epidermis containing Langerhans cells in response to allergens. Exposure of the reconstructed epidermis containing Langerhans cells to allergens for 24 h resulted in a CD86 mRNA over-expression while irritants had no effect. This over-expression was not detectable after 5 h (data not shown) or in Langerhans cell-free epidermis after 24 h. We observed a constitutive CD86 mRNA expression in untreated reconstructed epidermis containing Langerhans cells, but did not detect CD86 mRNA in the absence of Langerhans cells. Therefore, Langerhans cells are the unique source of CD86 mRNA and changes in its expression directly reflect the allergen-induced modifications in the Langerhans cells.

This 3-dimensional model is sufficiently sensitive to detect IL-1 β and CD86 responses induced by a weak sensitizer. Such an *in vitro* model was lacking until now as Kimber underlined in his review (Kimber et al., 1999).

Inter-individual differences in the response of human blood-derived dendritic cells to allergens have been described (Pichowski et al., 2001). We also noted a CD34⁺ cell donor dependent reactivity after challenge with allergens. The allergen-induced morphology variation was observed for 88% of donors. The allergen-induced mRNA IL-1 β and CD86 over-expressions were observed for 58% of donors. Higher response rates and probably better representative data were obtained using CD34⁺ progenitors pooled from different donors as a source for the Langerhans cells.

SSR exposure of the reconstructed epidermis induced within 24 h a remarkable decrease in the number of the Langerin-positive cells and a reduction of the dendrites in the remaining cells. These UV-induced effects resemble very much those observed *in vivo* (Cooper et al., 1992; Elmetts et al., 1992; Ullrich, 1995; Neale et al., 1997; Kelly et al., 2000; Seit   et al., 2003) and described as

UV-induced immunosuppression. It is interesting to note that the application of a broad-spectrum sunscreen before irradiation afforded complete protection of the integrated Langerhans cells against SSR-induced damages. Similar results about sunscreen protection against UV-induced immunodamages have been reported *in vivo* (Moyal and Fourtanier, 2001; Dumay et al., 2001).

In conclusion, these data indicate that the reconstructed epidermis with integrated CD34⁺-derived Langerhans cells not only provides an excellent means to study *in vitro* Langerhans cell physiology, metabolism and degree of permeability of allergens (B  rard et al., 2003), but may also be used as an alternative approach to evaluate the sensitizing potential of native or formulated chemicals and the efficacy of sunscreens in the field of immunophotoprotection.

Acknowledgement

We thank Drs Cals-Grierson and Ferraris for their helpful advice in performing RT-PCR and Dr Fran  oise Rousset for interesting discussions. We gratefully acknowledge Novartis Pharma S.A. for supplying us with GM-CSF. This work was supported by a grant from the European Community (BIO4 CT 960086 biotech program).

References

- Aberer, W., Schuler, G., Stingl, G., H  nigsmann, H., Wolff, K., 1981. Ultraviolet light depletes surface markers of Langerhans cells. *Journal of Investigative Dermatology* 76, 202–210.
- 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. *European Journal of Immunology* 27, 3031–3038.
- Aiba, S., Manome, H., Yoshino, Y., Tagami, H., 2000. *In vitro* treatment of human transforming growth factor-  1-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.
- Bacci, S., Romagnoli, P., Streilein, W., 1998. Reduction in number and morphologic alterations of Langerhans cells after UVB radiation in vivo are accompanied by an influx of monocytoïd cells into epidermis. *Journal of Investigative Dermatology* 111, 1134–1139.
- Bérard, F., Marty, J.P., Nicolas, J.F., 2003. Allergen penetration through the skin. *European Journal Dermatology* 13 (4), 324–330.
- Caux, C., Dezutter-Dambuyant, C., Schmitt, D., Banchereau, J., 1992. GM-CSF and TNF- α cooperate in the generation of dendritic Langerhans cells. *Nature* 360, 258–261.
- Cooper, K.D., Oberhelman, L., Hamilton, T.A., et al., 1992. UV exposure reduces immunization rates and promotes tolerance to epicutaneous antigens in human: relationship to dose CD1a⁺DR⁺ epidermal macrophage induction and Langerhans cell depletion. *Proceedings of the National Academy of Sciences of the USA* 89, 8497–8501.
- Coutant, K.D., de Brugerolle de Fraissinette, A., 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. *Toxicological Sciences* 52, 189–198.
- Cumberbatch, M., Dearman, R.J., Kimber, I., 1997. Langerhans cells require signals from both tumour necrosis factor- α and interleukin-1 beta for migration. *Immunology* 92, 388–395.
- Cumberbatch, M., Griffiths, C.E.M., Tucker, S.C., Dearman, R.J., Kimber, I., 1999. Tumour necrosis factor- α induces Langerhans cell migration in humans. *British Journal of Dermatology* 141, 192–200.
- de Groot, A.C., Weyland, J.W., Nater, J.P., 1994. Unwanted Effects of Cosmetics and Drugs Used in Dermatology. Elsevier.
- Dumay, O., Karam, A., Vian, D., et al., 2001. Ultraviolet A1 exposure of human skin results in Langerhans cell depletion and reduction of epidermal antigen-presenting cell function: partial protection by a broad-spectrum sunscreen. *British Journal of Dermatology* 144, 1161–1168.
- Elmets, C.A., Vargas, A., Oresajo, C., 1992. Photoprotective effects of sunscreens in cosmetics on sunburn and Langerhans cell photodamage. *Photodermatology Photoimmunology Photomedicine* 9, 113–120.
- Enk, A.H., Katz, S.I., 1992. Early molecular events in the induction phase of contact sensitivity. *Proceedings of the National Academy of Sciences of the USA* 89, 1398–1402.
- Fithian, E., Kung, P., Goldstein, G., Rubinfeld, M., Fenoglio, C., Edelson, R., 1981. Reactivity of Langerhans cells with hybridoma antibody. *Proceedings of the National Academy of Sciences of the USA* 78, 2541–2544.
- Jellis, C.L., Wang, S.S., Rennert, P., Borriello, F., Sharpe, A.H., Green, N.R., et al., 1995. Genomic organization of the gene coding for the costimulatory human B-lymphocyte antigen B7-2 (CD86). *Immunogenetics* 42, 85–89.
- Kelly, D.A., Walker, S.L., McGregor, J.M., Young, A.R., 1998. A single exposure of solar simulated radiation suppresses contact hypersensitivity responses both locally and systemically in humans: quantitative studies with high-frequency ultrasound. *Journal of Photochemistry Photobiology B* 44, 130–142.
- Kelly, D.A., Young, A.R., McGregor, J.M., Seed, P.T., Potten, C.S., Walker, S.L., 2000. Sensitivity to sunburn is associated with susceptibility to ultraviolet radiation-induced suppression of cutaneous cell-mediated immunity. *Journal of Experimental Medicine* 191, 561–566.
- Kimber, I., Dearman, R.J., Cumberbatch, M., Huby, R.J.D., 1998. Langerhans cells and chemical allergy. *Current Opinion of Immunology* 10, 614–619.
- Kimber, I., Pichowski, J.S., Basketter, D.A., Dearman, R.J., 1999. Immune response to contact allergens: novel approach to hazard evaluation. *Toxicology Letters* 106, 237–246.
- Kimber, I., Basketter, D.A., Gerberick, G.F., Dearman, R.J., 2002. Allergic contact dermatitis. *International Immunopharmacology* 2, 201–211.
- Krasteva, M., Kehren, J., Ducluzeau, M.T., Sayag, M., Cacciapuoti, M., Akiba, H., et al., 1999. Contact dermatitis I. Pathophysiology of contact sensitivity. *European Journal of Dermatology* 1, 65–77.
- Kripke, M.L., Morison, W.L., 1985. Modulation of immune function by UV radiation. *Journal of Investigative Dermatology* 85 (suppl.), 62S–66S.
- Larsen, C.P., Steinman, R.M., Witmer-Pack, M., Hankins, D.F., Morris, P.J., Austyn, J.M., 1990. Migration and maturation of Langerhans cells in skin transplants and explants. *Journal of Experimental Medicine* 172, 1483–1493.
- Manome, H., Aiba, S., Tagami, H., 1999. Simple chemicals can induce maturation and apoptosis of dendritic cells. *Immunology* 98, 481–490.
- Matsunaga, T., Katayama, I., Yokozeki, H., Nishioka, K., 1998. Epidermal cytokine mRNA expression induced by hapten differs from that induced by primary irritant in human skin organ culture system. *Journal of Dermatology* 25, 421–428.
- Meunier, L., 1999. Ultraviolet light and dendritic cells. *European Journal of Dermatology* 9, 269–275.
- Moyal, D.D., Fourtanier, A.M., 2001. Broad-spectrum sunscreens provide better protection from the suppression of the elicitation phase of delayed-type hypersensitivity response in humans. *Journal of Investigative Dermatology* 117, 1186–1192.
- Neale, R., Russell, A., Muller, H.K., Green, A., 1997. Sun exposure, sunscreen and their effect on epidermal Langerhans cells. *Photochemistry Photobiology* 66, 260–264.
- Pichowski, J.S., Cumberbatch, M., Dearman, R.J., Basketter, D.A., Kimber, I., 2001. Allergen-induced changes in interleukin-1 β (IL-1 β) mRNA expression by human blood-derived dendritic cells: inter-individual differences and relevance for sensitization testing. *Journal of Applied Toxicology* 21, 115–121.
- Rambukkana, A., Pistor, F.H.M., Bos, J.D., Kapsenberg, M.L., Das, P.K., 1996. Effects of contact allergens on human Langerhans cells in skin organ cultures: migration, modulation of cell surface molecules, and early expression of interleukin-1 beta protein. *Laboratory Investigative* 74, 422–436.
- Reutter, K., Jäger, D., Degwert, J., Hoppe, U., 1997. In vitro model for contact sensitization: II. Induction of IL-1 β mRNA in human blood-derived dendritic cells by contact sensitizers. *Toxicology in vitro* 11, 619–626.
- Régner, M., Staquet, M.J., Schmitt, D., Schmidt, R., 1997. Integration of Langerhans cells into a pigmented reconstructed human epidermis. *Journal of Investigative Dermatology* 109, 510–512.
- Rheinwald, J.G., Green, H., 1975. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. *Cell* 6, 331–344.
- Rougier, N., Redziniak, G., Mougin, 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.
- Rousset, F., Verda, D., Arrighi, J.-F., Péguet-Navarro, J., Cottin, M., 2000. A strategy for the prediction of skin sensitisation: an alternative to animal testing. In: Balls, M., van Zeller, A.-M., Halder, M.E. (Eds.), *Developments in Animal and Veterinary Sciences—Progress in the Reduction, Refinement and Replacement of Animal Experimentation*. Elsevier, pp. 635–642.
- Ryan, C.A., Hulette, B.C., Gerberick, G.F., 2001. Approaches for the development of cell-based in vitro methods for contact sensitization. *Toxicology in vitro* 15, 43–55.
- Schempp, C.M., Dittmar, H.C., Hummler, D., Simon-Haarhaus, B., Schulte-Mönting, J., Schöpf, E., et al., 2000. Magnesium ions inhibit the antigen-presenting function of human epidermal Langerhans cells in vivo and in vitro. Involvement of ATPase, HLA-

- DR, B7 molecules, cytokines. *Journal of Investigative Dermatology* 115, 680–686.
- Seité, S., Zucchi, H., Moyal, D., Tison, S., Compan, D., Christiaens, F., et al., 2003. Alterations in human epidermal Langerhans cells by UV radiation: quantitative and morphological study. *British Journal of Dermatology* 148, 291–299.
- Stoitzner, P., Holzmann, S., McLellan, A.D., Ivarsson, L., Stössel, H., Kapp, M., et al., 2003. Visualization and characterization of migratory Langerhans cells in murine skin and lymph nodes by antibodies against langerin/CD207. *Journal of Investigative Dermatology* 120, 266–274.
- Strobl, H., Riedl, E., Scheinecker, C., Bello-Fernandez, C., Pickl, W.F., Rappersberger, K., et al., 1996. TGF- β 1 promotes in vitro development of dendritic cells from CD34⁺ hematopoietic progenitors. *Journal of Immunology* 157, 1499–1507.
- Tinois, E., Tiollier, J., Gaucherand, M., Dumas, H., Tardy, M., Thivolet, J., 1991. In vitro and post-transplantation differentiation of human keratinocytes grown on the human type IV collagen film of a bilayered dermal substitute. *Experimental Cell Research* 193, 310–319.
- Tinois, E., Gaetani, Q., Gayraud, B., Dupont, D., Rougier, A., Pouradier Duteil, X., 1994. The Episkin model: successful reconstitution of human epidermis in vitro. In: Rougier, A., Goldberg, A.M., Maibach, H.I. (Eds.), *In Vitro Toxicology—irritation, Phototoxicity, Sensitization*. Mary Ann Liebert, New York, pp. 133–141.
- Tuschl, H., Kovac, R., 2001. Langerhans cells and immature dendritic cells as model systems for screening of skin sensitizers. *Toxicology in vitro* 15, 327–331.
- Ullrich, S.E., 1995. Modulation of immunity by ultraviolet radiation: key effects on antigen presentation. *Journal of Investigative Dermatology* 105, 30s–36s.
- Ullrich, S.E., Kim, T.H., Ananthaswamy, H.N., Kripke, M.L., 1999. Sunscreen effects on UV-induced immune suppression. *Journal of Investigative Dermatology* 4, 65–69.
- Valladeau, J., Duvert-frances, V., Pin, J.J., Dezutter-Dambuyant, C., Vincent, C., Massacrier, C., et al., 1999. The monoclonal antibody DCGM4 recognizes Langerin, a protein specific of Langerhans cells, is rapidly internalized from the cell surface. *European Journal of Immunology* 29, 2695–2704.
- Valladeau, J., Ravel, O., Dezutter-Dambuyant, C., Moore, K., Kleijmeer, M., Liu, Y., et al., 2000. Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. *Immunity* 12, 71–81.