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

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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-1β and CD86 mRNA over-expressions were detected in the reconstructed epidermis. A topical treatment with TNF-α or IL-1β 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.

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 allergens 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-1β 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.

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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-1β/CD86 mRNA expressions) to topically applied allergens and allergen-inducible cytokines. We also evaluated the immuno-photo-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 Fourtanier, 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 cm^2). 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 μg/ml transferrin, 2×10^{-9} M triiodothyronine, 1.8×10^{-4} M adenine and 5 μg/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 μm) 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 μl spread on the 1.1 cm^2 area using a curved Pasteur pipette).

TNF-α and IL-1β were purchased from R&D Systems (Abingdon, UK). Contact allergens: dinitrofluorobenzene (DNFB), oxazolone, *p*-phenylenediamine (pPD), $NiSO_4$, 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 $NiSO_4$) were solubilized in acetone/olive oil 4:1, while irritants and $NiSO_4$ 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, Boerhinger Mannheim, Germany). Total RNA was extracted with the Trizol® reagent (Life Technologies/GibcoBRL, Cergy Pontoise, France). First strand cDNA was synthesized from 1 μg RNA using an oligo$(dT)_{18}$ primer (First-strand cDNA synthesis kit, Amersham Pharmacia Biotech, Buckinghamshire,