

Langerhans Cells Integrated into Human Reconstructed Epidermis Respond to Known Sensitizers and Ultraviolet Exposure

To the Editor:

In 1997, we reported the first successful integration of human epidermal Langerhans cells into a reconstructed human epidermis (Régnier *et al*, 1997). All experiments were conducted with the proper institutional approval. After considerable effort, we succeeded in transferring the initial protocol to an epidermal model that has the potential to be produced at an industrial level. Within the epidermis, reconstructed on the collagenic Episkin dermal support, the CD34⁺-derived dendritic Langerhans cells are located in the suprabasal cell layers at a density (30,000 cells per cm²) comparable to that of normal human skin (Schempp *et al*, 2000). Advantageously to skin organ cultures, where Langerhans cells emigrate spontaneously from the epidermis (Larsen *et al*, 1990), they remained resident cells. As in the initial model, the integrated Langerhans cells exhibit all characteristic features of epidermal Langerhans cells such as Birbeck granules and CD1a surface antigens (Régnier *et al*, 1997).

In vivo, capture of low-molecular-weight allergens bound to epidermal proteins induces activation and migration of epidermal Langerhans cells to the regional lymph nodes where they present the processed antigen to naïve T cells (sensitization phase). This phase is characterized by morphologic, phenotypic, and functional modifications of the Langerhans cells, e.g., the upregulation of costimulatory molecules such as CD86 at their surface.

Several reports have described how ultraviolet (UV) exposure of the skin induces a phenomenon generally described as immunosuppression implicating directly the epidermal Langerhans cells (Kripke and Morison, 1985; Ullrich, 1995; Kelly *et al*, 1998). Immunosuppression after UV exposure is characterized by a reduction in the number of epidermal Langerhans cells, an alteration of their morphology, and a depletion of certain cell surface markers (Aberer *et al*, 1981; Bacci *et al*, 1998).

To assess the reactivity of the integrated Langerhans cells and to compare it to their *in vivo* response, we exposed the reconstructed epidermis to some known allergens, the irritant SLS, and solar simulated irradiation.

Reconstruction of the human epidermis containing Langerhans cells was achieved by co-seeding CD34⁺-derived dendritic cells (5×10^4 CD1a⁺) with 4×10^5 heterologous keratinocytes onto the Episkin support (1.3 cm²). Cells were submerged for 3 d in Dulbecco's modified Eagle's medium/Ham F₁₂ (3:1), containing 10% fetal bovine

serum, 10 ng per mL epidermal growth factor, 400 ng per mL hydrocortisone, 10^{-6} M isoproterenol, 5 µg per mL transferrin, 2×10^{-9} M triiodothyronine, 1.8×10^{-4} M adenine, and 5 µg per mL insulin. To induce epidermal differentiation, the reconstructs were put for 10 d at the air-liquid interface in culture medium without isoproterenol, transferrin, triiodothyronine, and adenine.

As shown in Fig 1, the human reconstructed epidermis on the Episkin support (Fig 1a) exhibits a well-stratified epidermis with Langerhans cells located in its suprabasal layers (Fig 1b). Immunostaining of the Langerhans cells in the respective epidermal sheet reveals a dense network of equally distributed Langerhans cells (Fig 1c). As reported earlier for the reconstructed epidermis on de-epidermized epidermis (Régnier *et al*, 1997), Langerhans cells do not emigrate spontaneously from the epidermis as reported in cultured skin explants (Larsen *et al*, 1990).

Exposure of the reconstructed skin to the known sensitizers dinitrofluorobenzene, NiSO₄, the irritant SLS, and solar simulated irradiation (Fig 2) affects the integrated Langerhans cells in a similar way as described for their *in vivo* counterparts. Topically applied sensitizers dinitrofluorobenzene and NiSO₄ (Fig 2b,c) induced a reduction in the number of Langerhans cells and affected their characteristic dendritic morphology, whereas the irritant SLS induced no visible changes, (Fig 2d) confirming the observations made by Aiba *et al* (1997) that dendritic cells respond differently to haptens and irritants. The same holds true for the upregulation of the surface antigen CD86 (Aiba *et al*, 2000) observed in the reconstructed epidermis after exposure to haptens (results not shown). In a separate set of experiments (not shown) we ensured that the upregulation of CD86 is specific for the integrated Langerhans cells, as keratinocytes do not express CD86 under our experimental conditions.

The most dramatic changes were observed after exposure of the reconstruct to solar simulated irradiation. A dose of 8.4 J per cm² solar simulated irradiation, equivalent to a sun exposure of about 1 h, induced not only a remarkable reduction in the number of langerin-positive cells, but also changed the morphology of the remaining Langerhans cells from a dendritic to a round cell type (Fig 2e). These UV-induced changes resemble very much those observed *in vivo* (Cooper *et al*, 1992; Neale *et al*, 1997), which are described to be closely related to UV-induced immunosuppression (Kelly *et al*, 1998). Interestingly, application of a broad-spectrum sunscreen before

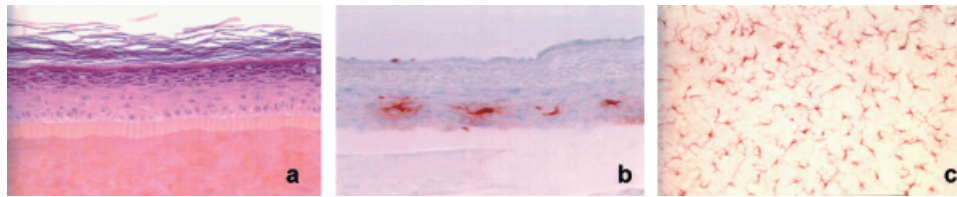


Figure 1

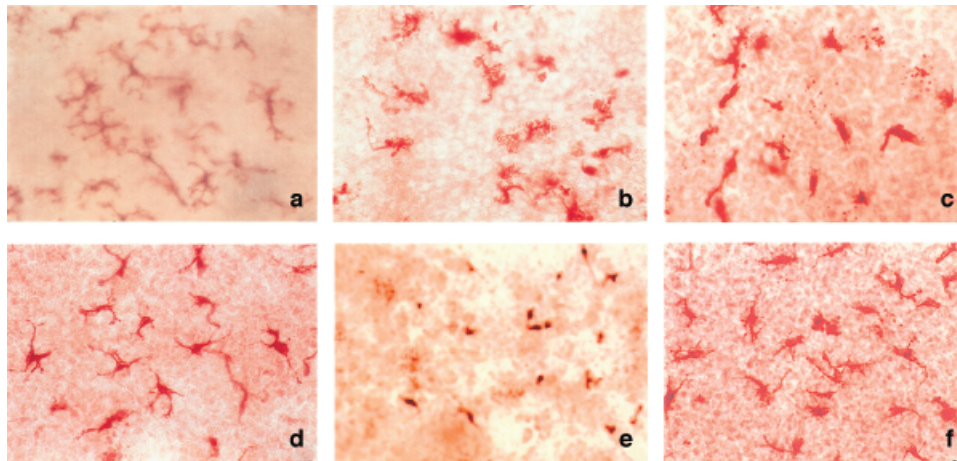


Figure 2

irradiation afforded, as already described *in vivo* (Dumay *et al*, 2001; Moyal and Fournanier, 2001), complete protection of the Langerhans cells (Fig 2f).

In conclusion, the above-described results, even though preliminary and incomplete, clearly show that the reactivity of the Langerhans cells integrated into a reconstructed epidermis is comparable to *in vivo*. Beside its potential to be produced at an industrial level and to serve some day as an alternative model for estimating the sensitizing potential of new chemicals, it also provides an excellent research tool to study Langerhans cell biology *in vitro*. This letter is also intended as an invitation for laboratories specialized in the field of immunology, in particular that of the skin, to use and further validate this model by generating new results and insights into epidermal Langerhans cell physiology.

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