Physiopathology and Organotypic Cultures of Human Keratinocytes

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The use of cultured skin substitutes in the study of skin physiopathology and pharmacology is expanding. Recently, a technique consisting of culturing human epidermal cells on air-exposed de-epidermalized dermis has been proposed [1]. In these conditions, a stratified epidermis was obtained after 1 week of culture [2, 3]. Its architecture closely resembled epidermis in vivo, with defined epidermal layers. Proliferation was largely restricted to the basal layer. The cell cycle of the germinative cells, measured by a percentage labelled mitoses technique was about 15 h. The transit time of cells in the differentiated compartment, evaluated by the appearance of labelled cells in the granular layer, was 4 days [4]. Despite this high cell renewal, normal cell differentiation was maintained with expression of various keratinization markers and formation of a stratum corneum without parakeratosis. This suggests that in psoriasis, cell differentiation is an intrinsic abnormality rather than a simple failure to complete the normal process of keratinization.

This complex culture system may be used for the study of cytopathic effects of virus infection and the establishment of productive in vitro systems, i.e. for the clinically important human papilloma virus (HPV). Earlier studies demonstrated the difficulties to study HPV in vitro [5]. This was probably due to the incomplete maturation that was obtained with conventional culture techniques. The problem was obviated by the use of two steps in the procedure [6].

First, primary cultures of human keratinocytes were inoculated with HPV1. Then, upon transfer to dead de-epi-

dermalized dermis and growth at the air-liquid interface, HPV1 DNA amplification was found to take place in the reconstituted epidermis, being detectable from 7 days after the transfer and at least 10 days thereafter. However, in contrast with their capacity for HPV DNA synthesis, the cultures did not appear to produce detectable amounts of viral capsid proteins.

The same culture system can be used for studies that require the retention of a pathological phenotype in vitro [7]. For example, a reproduction of the epidermal lesions of Hailey-Hailey disease was obtained, suggesting that in this disease the epidermis is the site of the defect leading to acantholysis without any dermal contribution [8]. On the contrary, with the same technique, psoriatic lesions lost their specific phenotype and became similar to normal keratinocyte cultures [Willaert, Heenen, unpubl. results].

Organotypic cultures are also valuable in vitro models for the study of how epidermal differentiation and tissue homeostasis is regulated [9, 10], of how the barrier function is reconstituted [11] and of the toxic effects of chemicals [12] or drugs [13].

Finally, they can be used as a model of skin re-epithelialization, in particular to study the effects of electrical stimulation on epidermal healing. In a recent study, low-frequency alternating current had no effect of the growth rate of keratinocytes but seemed to enhance differentiation [Hinsenkamp, Heenen, unpubl. results]. This needs to be confirmed by additional experiments.

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