Psoriatic in vitro epidermis
A human tissue culture model for testing cosmetical and medical skin care products

CLAAS RÜFFER
Dermatest GmbH, Engelstrasse 37, Münster, 48143, Germany

KEYWORDS: Psoriasis, in vitro epidermis, FTM, Th17, Th1, IL-22, skin barrier.

ABSTRACT: The very complex nature of psoriasis pathogenesis and its course of disease mean an outstanding challenge for the development of reliable biological in vitro systems. Recent progress in psoriasis research has led to the development of new REACH-compatible in vitro Epidermis Models. Dermatest GmbH utilizes a cytokine-transformed in vitro 3D model that mirrors characteristic epidermal aspects of psoriasis. This model can be used to test topical treatments and skin care products in their effectiveness to restore lost skin barrier properties.

INTRODUCTION

Psoriasis is a common, chronic skin disease with a largely unknown pathophysiological mechanism, which affects approximately ten million people in the USA and European Union alone [6, 10]. It is characterized by recurrent episodes of red, scaly, raised skin plaques that develop within seemingly normal skin. Histological analysis reveals a vast infiltration of immune cells including T-lymphocytes, dendritic cells (DCs), macrophages, and granulocytes in the dermis and epidermis. Common skin symptoms are itching, soreness, and bleeding [10]. The disease adversely affects psychological and social functioning, and affected persons have a substantial loss of quality in life [9]. Biological models have a tremendous meaning for psoriasis research and treatment. Despite the great medical need, current psoriasis treatment options are limited, which is at least in part due to our restricted knowledge of its pathogenesis. In recent years substantial advances have been made in elucidating the molecular mechanisms of psoriasis [10, 12]. However, major issues including the primary nature of the disease, its initiation, progression, and response to therapies remain unresolved [6]. Never the less, recent progress in the understanding of the molecular and immunological basis of psoriasis has lead to an improved insight into disease mechanisms and new therapeutic approaches [6]. This great progress was predominantly possible due to the exhaustive use and investigation of available and novel in vivo and in vitro models of psoriasis, which cover a broad spectrum of disease relevant but also disputable aspects of the psoriasis pathology [4]. The complex nature of Psoriasis challenges identification and development of adequate biological models. With the exception of a few sporadic cases in primates, psoriasis is unique to humans [4]. This fact makes it exceptional difficult in any respect to find an ideal single biological model system which reflects all major sets of psoriatic features. The vast majority of today available systems are animal models of psoriasis, which usually provide only an approximation of the disease and are not conform to REACH legislations [4]. Main types of in vivo animal models usually rely on mice as hosts and are of highly diverse quality. Some genetic models are based on less defined spontaneous mutations others on genetic engineering [4]. More promising alternatives are so called induced models, where psoriasis involved human skin is transplanted on or diseased human immune components are transferred to mice as hosts [4]. Such exclusive, costly and delicate animal models can ideally be utilized as promising tools for medical and pharmaceutical research laboratories. Therefore novel, easy accessible, uncomplicated, REACH-compatible human in vitro test systems are badly needed, especially for the purposes of the developing and testing cosmetic industry. In vitro models of psoriasis are still quite limited in information but their use is continuously increasing. Despite the fact that in vitro model systems do not represent the whole complexity of real skin and still lack immune cells and blood vessels, in vitro reconstituted human epidermal and full thickness culture model systems have become a serious alternative to animals [4]. Epidermal keratinocytes are grown to the air-liquid interface and differentiate and stratify to mimic the morphology of normal stratified squamous epidermis. First attempts to construct an epidermal in vitro disease model of psoriasis were made by utilizing skin cells from freshly isolated acute lesional and from not involved psoriatic skins [2]. Limited numbers of donors and the high variability of results made this approach quite inefficient. At last, recent novel scientific findings revealed important new insights into the molecular mechanisms of the cellular cross-talk between epidermal and immune cells, which has shown to be responsible for the psoriatic epidermal transformation and plaque development [11]. This revised knowledge of psoriasis biology has been continuously transferred into designs of new and more reliable in vitro models. Some of them have already been successfully applied as tools for studying epidermal aspects of psoriasis [7]. Pro-inflammatory cytokines, capable to transform the morphological status of epidermal differentiation, can be used to build in vitro psoriatic epidermis models. Psoriasis most prominent feature is the very characteristic pathogenic skin phenotype. Evolution of a psoriatic lesion is based on a complex interplay between environmental and genetic factors that sets the scene for disease initiating events [6]. A cascade of events leads to activation of immune cells as dendritic and, in turn, the generation of effector T-lymphocytes, which emigrate to and reside in skin tissue. Cross-talk between epithelial cells and immune cells shapes and maintains the inflammatory milieu [12]. Cytokines, small cell-signalling protein molecules, play a decisive role in the communication between cells. In psoriasis keratinocytes are direct targets for an exclusive set of T-lymphocyte specific cytokines [12].
They affect the regulation of keratinocyte’s biological properties such as secretion of signalling and messenger molecules but also their differentiation and migration capacities (12). A whole set of immune cell specific inflammatory and pro-inflammatory molecules have been well characterized in lesional psoriatic skin and also in sera of psoriatic patients (1). In the past psoriasis was described as a Th1 (helper T-cell 1) type mediated inflammation. Novel findings approved that another subtype of T-lymphocytes, namely Th17, is highly involved in psoriasis development (9, 15). Interestingly the whole bandwidth of characterized Th1 and Th17 released cytokines, together with other disease-relevant cytokines and growth factors (e.g. IL-20, IL-17A, OSM, INF-γ, IL-1α and TNF-a) can ideally be used to build reconstructed in vitro epidermal models of psoriasis with normal and pathological keratinocytes as cell source (7, 11). Recent scientific findings describe a novel cytokine of the Interleukin-10-family, namely IL-22, which is also produced by Th17 lymphocytes in psoriasis affected skin (3, 14). In strong contrast to other disease relevant cytokines, IL-22 can comparably be detected in lesional psoriatic skin and in blood sera of diseased patients in significant levels (15). Interestingly, sera levels of IL-22 seem to correspond ideally to the stage of disease severity. For IL-22 it is strongly supposed to exclusively stimulate skin keratinocytes, which leads to the well described cutaneous phenotype of psoriasis (15). These findings have been confirmed in vitro. IL-22 successfully transformed regular three-dimensional organic skin tissues into epidermal equivalents revealing a distinct psoriasis-like morphology (4). In combination with transforming IL-22 other (pro)-inflammatory cytokines and growth factors (OSM, IL-17A, IL-1α, INF-γ, TNF-a etc.) can be used in vitro to generate a synergistic, psoriasis-like inflammatory milieu (7).

**MATERIAL AND METHODS**

**In vitro culture**

Phenion® organotypic skin models (FTM’s) were purchased from Henkel AG at All-day 10 and cultivated in serum-free All-medium as follows: DMEM with Glutamax/HAM’s F12 medium (3:1) supplemented with 1.6 mg/ml of bovine serum albumin, 0.2 mg/ml hydrocortisone, 0.12 IU/ml of insulin, 1mM ascorbic acid 2-phosphate (all purchased from Sigma, Germany), 100 IU/ml of penicillin and 100 mg/ml of streptomycin (K.R. Mewes et al., 2007). Epidermal IL-22 in vitro conditioning started at ALI (air-liquid interface)-day 10 when the 10 ng/ml of Interleukin-22. (R&D-Systems) was added to the culture medium. IL-22 containing culture media were refreshed each day. Full transformation was observed at transformation day 6.

**Topical treatment**

At All-day 11 FTM’s were thinly creamed once with 2 ml of Calcipotrol cream from Hexal and incubated for 6 to 7 days under optimal cell culture conditions.

**Histological analysis**

From the centre of each FTM 13 identical 4 mm biopsies were taken and fixed in 10 percent Formalin (Sigma) and embedded in paraffin. Further histochromotical routine HE and PAS stainings were performed on 5-mm sections. For immunohistochemistry (IHC), 5-mm sections on glass were deparaffinized and processed for anti-S100A7 (Imgenex; clone 47C1068) as described in Sa et al., 2007, (14). Histological stainings and skin biopsies have been obtained from the hospital and poli-clinic of skin diseases in Muenster, NRW, Germany, Department of Dermatology and Venerology.

**RESULTS**

Dermatest GmbH utilizes a Th17/Th1-cytokine transformed Phenion® Full thickness Model.

**IL-22 and in vivo psoriatic skin**

In vivo epidermal manifestation of patients was compared to the in vitro situation. The disease is usually manifested as raised, well-demarcated, erythemous oval plaques with adherent silvery scales. The scales are the result of a hyperproliferative epidermis. The hyperproliferation leads to a premature maturation of keratinocytes and an incomplete cornification that comes along with the retention of nuclei in the stratum corneum (parakeratosis) (10, Figure 1B). In vivo studies revealed that IL-22 is involved in the induction of lesional skin (5, 11; Figure 1B).

**IL-22 mediates morphological transformation of in vitro skin models**

Full thickness skin models, which were cultivated with IL-22, recapitulated the different morphological stages of epidermal...
tissue transformation usually seen in vivo during acute psoriatic phases (Figure 1B). The first morphological stages of epidermal transformation during acute psoriasis are characterized through a visible numeric reduction of granular cell layers (hypogranularity) and regular cornified (orthokeratotic) cell layers (10). Both are first signs of diminishing keratinocyte differentiation. At later stages of IL-22 action in vitro epidermis nicely shows the characteristic morphology of lesional skin. The transformed in vitro model reveals a massively thickened epidermis (acanthosis) with numerous layers of immature not fully differentiated, immature cornified layers, commonly marked as plaque (Figure 1A).

Topical Calcipotriol-treatment of IL-22 transformed skin models

Therefore, in vitro transformed psoriatic epidermis has been treated with a topical Calcipotriol containing cream in order to test the responsiveness of the organic test model. Calcipotriol is a synthetic Vitamin-D derivative, which is a well-proven, highly effective topical treatment against psoriatic lesional skin (8). Although its concrete mechanism of action is still incompletely resolved, Calcipotriol forces the hyperproliferative psoriatic epidermal cells back into differentiation and restores the integrity of skin’s barrier relevant structures. The comparative histological analysis of Calcipotriol-treated and untreated in vitro psoriatic epidermis has been performed. In a histochemical analysis (Figure 2, A-C and a-d), characteristic morphological aspects of skin barrier-relevant structures were visualized in order to examine signs of modified epidermal differentiation. Granular and cornified cell layers form the backbone of skin barrier-relevant structures in epidermis (13). Morphologically flat and longish appearing granular cells contain visible vesicular keratohyalin bodies and form the uppermost layers of the viable, highly differentiated epidermis (Figure 2, c-d, arrow). In contrast to regular polarized epidermis, both morphologically characteristic skin barrier structures are absent in IL-22 transformed in vitro epidermis (Figure 2, a, arrow) but can be restored through Calcipotriol action (Figure 2, b, arrow). This result suggests that Calcipotriol managed to at least some extent to overcome Interleukin-driven dysregulation of terminal differentiation in vitro.

DISCUSSION

The future of in vitro skin-specific disease models has just begun. It has been shown, that psoriasis is an immune-mediated disease. Targeted treatments against immunological cells or principal inflammatory mediators lead to its near-complete remission (10). A genetic basis in psoriasis is undisputed, but many of the causative genes remain to be identified (6). In consequence many essential molecular aspects of psoriasis pathogenesis are still completely unresolved and therefore designs of relevant and reliable in vitro models of psoriasis are hindered (4). Furthermore, the sheer complexity of psoriasis pathogenesis makes its all-embracing in vitro reconstruction almost impossible (4). Novel developed Full Thickness Skin Models exhibit a fully differentiated epidermis on top of a dermis-equivalent with integrated immune cells, as macrophages etc., or integrated vascular system. These new models could be used in future to test dermal and systemic endpoints (allergic potential, wound healing, inflammation, etc.) in combination with epidermal endpoints (4). Unfortunately, these model systems are still very experimental and far from being as complex as potentially needed to mirror all essential aspects of psoriasis. Never the less, recent scientific findings clarified very fundamental molecular mechanisms of psoriasis plaque formation. This knowledge has been successfully transferred into the design of reliable epidermal in vitro models of psoriasis, which most importantly, nicely respond to common, available topical treatments. Psoriasis treatments can be distinguished by their medical approach. They can target the epidermal alterations in order to restore lost skin barrier properties directly or they counter the more causative inflammatory events inside the psoriatic skin (10, 12). Cytokine transformed in vitro epidermal models can be ideally used as test platform to test cosmetic and medical products for their ability to restore lost epidermal polarization and skin barrier properties. It can definitely be concluded that epidermal in vitro models of psoriasis are useful and reliable tools to study efficacy of novel treatments or skin care cosmetics designed for topical skin management of mild forms and eczema-free intervals of psoriasis.

REFERENCES AND NOTES

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SABINSA EUROPE GmbH
www.sabinsacosmetics.com
Tel: 0049 6103 270 1111
E-mail: sabinsa.europe@sabinsa.com