Skin organ culture:
A review

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SUMMARY

Short-term organ culture of skin explants is a useful model for research into various aspects of skin biology. The use of skin organ culture systems in defining factors which affect homeostasis in elucidating modulatory effects of biologic response modifiers, drugs and physical agents on the skin and in studying complex aspects of cutaneous biology in normal and diseased skin is reviewed. Our own data regarding organ culture of rat full-thickness skin explants have been presented in this review.

KEY WORDS

skin explants, tissue integrity, inflammatory mediators, drug effects, cell

Introduction

Since the development of organ culture technique (1) this approach has been used for identification of various mediators in cultures of synovial, articular and other tissues and granulomas. Skin is easily organ-cultured (2,3). In this system characteristics of skin are displayed more clearly comparing with cell-culture systems, as the architecture of the tissue remains intact and cell-cell interactions are relatively undisturbed. Therefore it is suitable for investigations of various aspects of skin function and biology. Various skin organ culture systems have been described depending on the source of skin specimens (neonatal or adult skin), the size of skin explant (1-9 mm ^{2} or 1cm) and culture conditions (choice of liquid media, total or partial submersion, freely floating or on various supports). By using these systems it is possible to study various aspects of both normal and pathological skin biology, *ex vivo* or *in vitro* entirely.

In this paper, we have reviewed the use of human and animal skin organ culture in assessing skin homeostasis and inflammatory/immune reactions, in evaluating effects of various agents on the skin and complex aspects of skin cell behavior involved in cell migraton. In addition, we have presented our own data obtained by the use of organ-cultured full-thickness rat skin explants.

Tissue integrity and architecture of skin in organ culture

In organ-cultured skin normal relationship between epithelial cells and between fibroblasts, epithelial cells

and extracellular matrix is preserved. Thus, organ cultures of human skin have been utilized to study normal growth and differentiation. The tissue cultured in a regular culture medium with nutrient mixtures for skin cell culture and growth supplements (epidermal growth factor, insulin, hydrocortisone, bovine pituitary extract) remains viable for a few days, but necrosis can usually be seen by week 1 (4,5). The major degenerative changes include epidermal necrosis, destruction of the basal layer and separation of the epidermis from underlying basal lamina, with loss of celularity and a breakdown of the extracellular connective tissue structures in the dermis. Experiments with organ cultures established from more than 70 adult human skin specimens indicated that, under appropriate conditions (i.e. the presence of serum-free, growth factor-free culture medium supplemented with exogenous calcium ions to a final concentration of 1.4 nM or 3 μM of retinoic acid, human skin can be kept in organ culture histologically normal in appearance and biochemically active for at least 12 days and up to 24 days (5). It was suggested that under these culture conditions production of growth factors required for the maintenance of homeostasis is favoured. The production of components of extracellular matrix (fibronectin and thrombospondin) by the organ cultured skin in the presence of 1.4 nM Ca++ was demonstrated also (6). As the maintenance of human skin explants in organ culture depends on conditions optimized for fibroblast proliferation and not on those optimized for keratinocyte growth, a critical role for fibroblast viability and function was proposed in maintaining cutaneous homeostasis (7). This organ culture model is thus suitable for studying mechanisms responsible for the maintenance of normal skin homeostasis. It has also proved to be a useful tool for studying skin response to injury and pathophysiolgical mechanisms of altered differentiation and proliferation in psoriasis. Organ culture of psoriatic skin was recommended as a useful tool for deciphering pathophysiological mechanisms of aberrant keratinocyte proliferation and its possible modulation *in vitro* (8). Maintenance of psoriatic lesional skin in organ culture (9) as well as mimicking the features of psoriatic skin in organ cultures of normal skin by exposing it to a growth factor-enriched medium (8), provide an experimental approach for delineation of factors which are critical to the maintenance of psoriatic lesion. By this approach the involvement of epidermal growth factor (EGF) in maintaining the psoriatic phenotype was demonstrated in vitro using an antibody to the human EGF receptor (8). The effects of a vitamin A derivative, tretinoin were followed in organ cultures of psoriatic lesional skin. It was shown that keratinization of the involved psoriatic epidermis is sensitively controlled by vitamin A, with granular layers appearing in the absence of the vitamin or disappearing with relatively low concentration of the vitamin (10).

By using human skin organ cultures, the role of retinoids in skin homeostasis has been extensively studied.

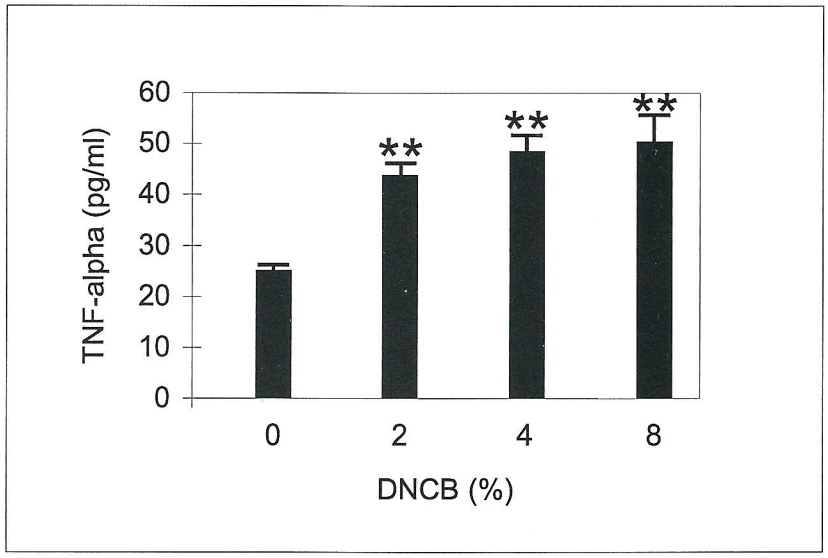
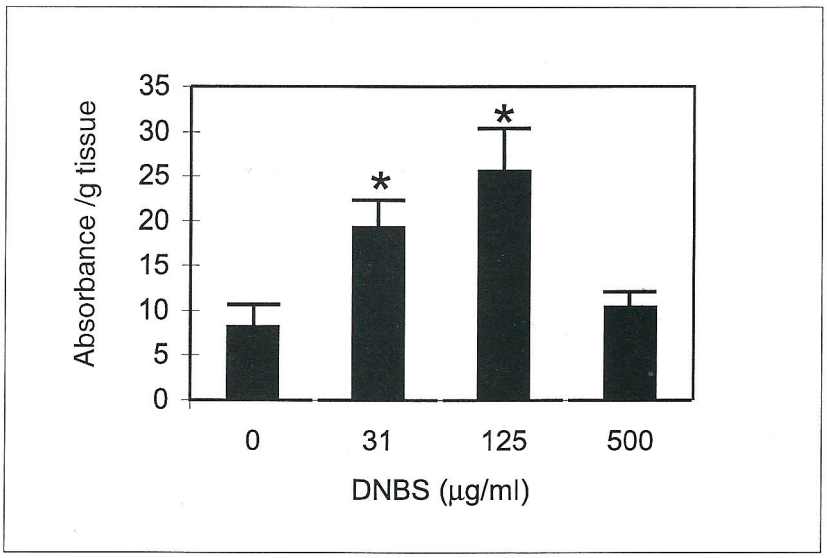


Fig 1. TNF-α levels in culture fluid of organ-cultured rat ear skin following topical application of various doses of DNCB. Values are expressed as pg TNF-α/ml of culture fluid, determined by a commercial ELISA test. Significance at * 0.05 or ** 0.01 vs vehicle treatment.

Fig 2. Tetrazolium reducing capability of skin explants cultured in the presence of DNBS. Results are expressed as absorbance of extracted formazan product. Significance at 0.05 vs control skin explants.



It was shown that retinoids maintained viability of skin organ cultures, but the failure to maintain normal epidermal differentiation was noted (5). An investigations into the effects of retinoic acid (RA) on epidermal homeostasis in a human organ culture system revealed the RA-induced expression of heparin-binding epidermal growth factor (Hb-EGF), a member of the epidermal growth factor family, which in turn might be responsible for the observed effect of the drug which promotes epidermal hyperplasia (11). Retinoic acid (RA) treatment of organ-cultured skin resulted in decreased cohesiveness and extensive acantholysis, accompanied, in some skin specimens, with separation of the corneal from the basal and suprabasal layers (5,6). The retinoid-induced loss of epidermal cohesion could be attributed to a reduced synthesis of components of extracellular matrix (6) and/or production of proteoloytic enzymes (urokinase and tissue plasminogen activator) in the presence of this agent (12). It was suggested from these studies that proteases might influence the structural integrity of the tissue. The epidermal-dermal junction of organ cultured skin was shown to be highly susceptible to neutral serine proteases derived from human skin, mast cells and polymorphonuclear leukocytes (13). Thus, in vitro culture of the skin in the presence of proteolytic enzymes or agents, which induce or facilitate their activity, might provide a clue to the involvement of proteinases in skin diseases.

The involvement of proteinases in the altered skin integrity was suspected in early studies of skin explants cultured with proteinases or agents, which induced proteinase activity. A loss of epidermal cohesion was demonstrated in organ cultures of skin explants incubated with immunogobulin G (IgG) from pemphigus sera (14). As plasminogen enhanced the ability of pemphigus IgG to cause acantholysis in organ culture, the involvement of plasminogen activator (PA) was indicated in the process (14). A recent study in the uPA knock-out (with targeted disruption of PA genes) neonatal mouse model of pemphigus (15) provides evidence that IgG from pemphigus vulgaris and pemphigus foliaceus cause the loss of cell adhesion directly via binding to desmogleins, interfering thus with their function, rather than indirectly by releasing proteases.

Binding of IgG from bullous pemphigoid sera for the basement membrane zone in a skin explant culture system has been regarded as a very sensitive method for the detection of bullous pemphigoid antibodies (16).

The development of epidermis under the influence of the thyroid hormone, gluccocorticoids and estrogen was followed in an explant model of fetal rat skin (17). The involvement of a variety of ligands, activators and nuclear receptors in the hormone's action was revealed on the basis of expression of structural proteins (profillagrin and loricrin) of the stratum corneum.

Organ culture as a means to study skin inflammation.

Cutaneous inflammation could be investigated in skin organ culture by monitoring the presence of various soluble biochemical and inflammatory/immunoregulatory mediators and expression of molecules relevant to inflammatory cell infiltrate formation. Culture fluids are being used to collect mediators released from skin explant for their identification. In this regard is illustrative a systematic study of dermal inflammation in rabbit skin following topical application of sulphur mustard, in which organ cultures of developed and healing lesions were established and mediators in the culture fluids of explants monitored (18). Organ-culturing of lesional biopsies enabled the authors to determine a local turnover of serum proteins within the lesions themselves, in contrast to most studies in which leakage of serum or removal of serum proteins from lesions was reported (19). High levels of serum proteins were found in acute inflammatory lesions with rapid turnover rate. It was further demonstrated that the serum is a major source of unbound extracellular protein within these lesions, serum proteinase inhibitors being a major defense against local damage by proteinases from serum, activated infiltrated leukocytes and skin resident cells (19). Lysosomal enzymes were identified among inflammatory mediators released in culture fluid of skin explants, with higher levels in the culture fluid from healing lesions and with polymorphonuclear leukocytes and fibroblasts identified histochemically as their source (20). By using an *in vitro* approach entirely (i.e. in vitro injury to human skin by sulfur mustard), histamine, prostaglandin E2 and plasminogen activator were identified as mediators which initiate the inflammatory response to this agent (21).

Skin organ culture is suitable for studying soluble mediators of cutaneous inflammation, cytokines. Both epidermal and dermal elements (keratinocytes, Langerhans cells, fibroblasts, and endothelial cells) are the source of endogenous cytokines collected in organ culture fluids (22). Data obtained by organ-culturing the skin from various conditions of trauma implied that a skin injury might evoke a local response characterized by inflammation-related cytokine production. Significantly higher amounts of interelukin-6 (IL-6) were found in culture fluids of human skin specimens obtained from the operative wounds within a short time postoperatively, compared to the levels detected in

Table 1. Overview into the use of skin organ culture

PROCESS / FUNCTION	ORGANISM	MARKER FOLLOWED	REFERENCE
SKIN HOMEOSTASIS / GROWTH / DIFFERENTIATION			
Normal skin			
Maintenance in vitro	Human	Degenerative changes / histology	4,5
		ECM*	6
		Fibroblast growth / viability	7
Drug / biologic modifiers effects			
- retinoic acid	Human	- epidermal hyperplasia / cohesiveness	6, 11, 12
- proteinases	Human	- skin integrity	13
- antibodies: pemphigus serum	Human	- acantholysys	14
bullous pemphigoid serum	Human	- basement membrane binding	16
- hormones	Rat (fetal)	- epidermal development	17
Psoriasis lesional skin			
Maintenance in vitro	Human	- histology	8,9
Drug / biologic modifiers effects			
- EGF*	Human	- psoriatic phenotype	9
- tretinoin	Human	- epidermal keratinization	10
INFLAMMATION			
Inciting agent			
- Sulphur mustard	Rabbit	Soluble inflamatory mediators (serum proteins,lysosomal enzymes)	18,19,20,21
- Sulphur mustard	Human	Histamine,PGE_2, PA	21
- Operative injury	Human	Cytokines	23
- Thermal injury	Rat	Cytokines	24
- Contact allergen	Rat	Cytokines	This study
	Rat	Metabolic activity	This study
	Human	Cytokine mRNA	32
VARIOUS AGENTS EFFECTS			
- Cutaneous allergens	Human	Paranuclear vacuolization	36
	Human	Adhesive molecules	37
- Drugs	Human (FDE*)	Cytokine-mediated adhesive molecules	38
- UV	Human	Heat shock proteins	39, 40
	Human (vitiligo)	Morphological, biochemical (fair follicle)	41
- Cold	Human	Keratinocyte proliferation	42
- Ionizing radiation	Human	Adhesion molecule	43
CELL MIGRATION	Mouse	Appearance	44
	Human	Appearance	45
		Cell adhesion / aggregation	46,47
		HIV containment	48
		Phenotype	49, 50, 51
		Route of migration	52
	Human	Wound healing	53, 54

*ECM - extracellular matrix; EGF – epidermal growth factor; FDE – fixed drug eruption

fluids from uninjured skin either preoperatively or postoperatively (23). IL-6 secretion by skin explants could be stimulated during in vitro culture by egzogenous inflammation-related cytokines, tumour necrosis factor (TNF)-α and IL-1 and inhibited by corticosteroids, suggesting that a local skin cytokine response might be

influenced by inflammatory mediators from systemic microenvironment as well.

Organ culture of rat skin was employed in our laboratory in the *ex vivo* evaluation of local cytokine responses in an experimental model of thermal skin injury. Increased levels of TNF-α and IL-1 in culture fluids of skin explants indicated a cytokine response in the vital edge of the thermally injured skin early following the injury (24).

We employed organ culture of rat skin in studying skin response to a cutaneous allergen, dinitrochlorobenzene (DNCB) in a previously described experimentally induced contact hypersensitivity reaction (CHS) in rats (25). In this model of cutaneous inflammation/immunity, the reaction is generated (the induction phase) and expressed (elicitation phase) in the skin. Epicutaneous application of DNCB in the elicitation phase of CHS resulted in ear swelling, reflecting a local inflammatory response to hapten. The intensity of this response could be quantitated by the "ear swelling assay" which is a measure of the intensity of local inflammatory reaction in animal models of contact sensitivity. Recent data demonstrated the involvement of nitric oxide (NO) in the expression of CHS in mice and contribution of this inflammatory mediator to the ear swelling (26). Our preliminary data of *ex vivo* determination of levels of NO in culture fluids of the skin from ears following topical application of DNCB in the elicitation phase of CHS, demonstrated an increase in NO (approximately 5-fold), as estimated by nitrite levels in the Griess reaction (27).

The intensity of inflammatory reaction during the elicitation of CHS in rats was evaluated by determining the levels of TNF-α, an essential, primary cytokine involved in the expression of this experimental allergic reaction (28) in culture fluids of dorsal, cartilage free halves of ears, exposed to DNCB (Fig 1). Increased levels of TNF-α were noted with increasing doses of topically applied DNCB.

The local inflammatory/immune response to skin sensitizing chemicals is preceded by the "preimmunological" phase of response, characterized by oxidative activities of skin cells (29). We employed rat skin organ culture to assess the potential of the skin to generate reactive oxygen species in response to DNCB. The *in vitro* potential of the skin to generate reactive oxygen intermediates depends in part on the activity of tetrazolium-reducing respiratory burst oxidase (30) and could be determined via tetrazolium salts such is MTT (3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) to insoluble formazan products (31). The effect of dinitrobenzene sulfonic acid (DNBS), a water-soluble DNCB analog, on tetrazolium reducing capability of the skin in organ culture is presented in Fig 2. The increased reduction of MTT was shown in the presence of increasing doses of DNBS in culture. A decreased MTT reducing activity of skin specimens cultured with 500 μg DNBS/mL, probably reflects toxicity of DNBS, as only viable skin cells possess a MTT reducing capability. Additionally, skin organ culture enabled an analysis of early molecular events, including expression of messenger RNAs for inflammatory cytokines induced in human epidermis by contact allergens or irritants (32).

Cutaneous inflammation could also be evaluated by monitoring in situ (in an organ-cultured skin explant) expression of adhesion receptors on endothelial cells, leukocytes and keratinocytes, the crucial molecules for inflammatory leukocyte extravasation, migration and their interaction with resident cells (33). Modulation of adhesion molecule expression by inflammatory mediators such as cytokines may be studied in organ culture. Short-term organ culture of neonatal foreskin was used as a model to elucidate relative effects of IL-1, TNF-α and immune interferon (IFN-γ) on the inducibile expression of activation markers of microvascular endothelial cells (34). The endothelial cell response was demonstrated immunohistochemically during in vitro cultivation with the cytokines, localized predominantly to the postcapillary venules of the superficial vascular plexus, i.e. to those vessels most associated with inflammatory infiltration *in vivo*. The role of mast cell secretory activity in the initiation of cutaneous inflammation has been suggested by using a similar skin organ culture system. A rapid induction of endothelial cell activation antigen (ELAM, CD62E), a crucial endothelial cell molecule for leukocyte-endothelial cell adhesion, was demonstrated in the adjacent microvasculature following in vitro stimulation of mast cell degranulation in the perivascular space. The induction ELAM has been shown to be TNF-α- dependent, as no or only weak ELAM expression was shown in the presence of an anti-TNF antibody (35). Data obtained by this in vitro organ culture model highlighted the role of secretory activity of mast cells in cutaneous inflammation and established a potential link between mast cell-associated proinflammatory mediators (i.e. TNF-α) and the development of a cellular inflammatory response.

Evaluation of effects of chemical and physical agents in skin organ culture

Organ culture of skin explants is widely used as an approach to assess modulatory and/or adverse effects of various chemical and physical agents and biologic response modifiers. These effects may be studied both *ex vivo* or following an *in vitro* exposure of the skin, by monitoring changes in skin viability, tissue structure/

integrity and parameters of cutaneous inflammation.

Skin viability may be determined by changes in metabolic activity of skin explants measured by the enzymatically-mediated reduction of tetrazolium salts (31) or by observing paranuclear vacuoles (termed "storage-type") associated with gradual cell autolysis (36). The paranuclear vacuolization test has been recommended as a reliable test for evaluating dermatotoxic potential of topically applied chemicals (36).

Organ culture systems are particularly useful in tracing effects of various agents both of biological origin or drugs on diseased skin, where the expression of markers of inflammation was demonstrated in vivo. It was shown in organ cultures of the skin from atopic individuals that ELAM (CD62E) expression on the vascular endothelium, can be induced by *in vitro* exposing the skin specimens to cytokines or allergens (37).

A short-term organ culture of fixed drug eruption (FDE) lesional and nonlesional skin was employed to investigate the cellular and molecular events responsible for the observed skin inflammatory response to ingested drug (38). The obtained results indicated that the lesional keratinocytes and endothelium responded more rapidly and intensely than nonlesional skin to TNF-α and IFN-γ by expression of intercellular adhesion molecule (ICAM-1), a major activation antigen of keratinocytes in conditions of inflammation and a general adhesion molecule on activated endothelial cell. ICAM-1 expression on keratinocytes and endothelium induced by the causative drug alone was restricted to the FDE lesional skin organ cultures, which was abrogated by neutralization of TNF-α by anti-TNF-α antibody. Data, which demonstrated the drug-induced and cytokine-dependent expression of ICAM-1 in the lesional skin specimens, suggested that the induction of expression of this activation adhesion molecule could provide an initial localized stimulus for development of inflammatory response leading to activation of infiltrating leukocytes (38).

Skin response to different environmental stimuli, including various chemical and physical stressors has been assessed in organ-cultured skin. *In vitro* exposure of skin explants to azetidine carboxylic acid, sodium arsenite, cadmium as well as to UV -irradiation and heat resulted in the expression of heat shock protein HSP 72, an inducible member of the family of stress-induced molecules required for cell survival during and after stresses of variuos origin (39). As biologic effects of HSP were considered primarily in association with thermotolerance, and therapeutic hyperthermia and UV irradiation have been used for the treatment of various cutaneous diseases, organ-cultured skin is deemed a useful model for studying this class of cell defense proteins in human skin diseases. In addition, as there is an age-related decrease in the inducibility of HSP 72 in organ-cultured normal human skin (40), monitoring of the UV-induced expression of these molecules in organ cultures could be a useful additional approach in investigation of diminished ability of aged skin to respond to the adverse environmental conditions and to maintain homeostasis. The exposure of organ-cultured skin from the margins of vitiligo lesions *in vitro* to UV light, revealed morphological (dendricity) and biochemical (catechol oxidase and noradrenaline positivity) changes as features of UV responsiveness, pointing to the hair follicle as a specialized UV receptor in human skin (41).

Organ-cultured human skin has been regarded a useful model for evaluating the response of human skin to freezing (monitored by histology, immunolabelling and incorporation of radioactive label by proliferating cells) during the development of cryolesions as well as in elucidating the healing process of cryosurgery wounds (42). This culture system was used in studying the endothelial cell response to ionizing radiation through the assessment of adhesion molecule (VCAM-1, ICAM-1 and PECAM-1) expression in response to a clinically relevant dose range (43).

Organ culture as a model for studying cell migration

If explants of mouse (44) and human (45) skin are placed in organ culture, cells spontaneously migrate out into the culture medium surrounding the explant. The cell emigration was observed during 3 days, with highest numbers of migrated cells during the first 24-h of culture. Thereafter keratinocytes appear in the medium. Emigrants from human skin specimens comprise dendritic cells, lymphocytes and macrophages, dendritic cells being the most abundant among the migrated cells. Immunophenotyping of dendritic cells, which appeared in the medium during 3 consecutive days, demonstrated a high proportion of both epidermal ($CD1a^+$) and dermal ($CD1b^+$) dendritic cells (45). The migratory properties of dendritic cells interface with their capacity to initiate cutaneous immune responses through their extraordinary capacity to activate T cells. Among the skin cell emigrants, T cells could be found, predominantly belonging to the $CD4^+$ subset (the $CD4^+/CD8^+$ subset ratio is about 2:1), almost entirely with α/β T-cell receptor and with the phenotype of memory cells (46). The population of cutaneous emigrant leukocytes is highly viable (more than 95%) and devoid of contaminant keratinocytes as seen using the conventional skin disaggregation enzyme methods, with an average yield of 60000 leukocytes/cm^2 (43). Thus skin organ culture systems make accessible both T lymphocytes and dendritic cells in a highly enriched form enabling their

study. Cutaneous T cell migrants were found in stable aggregates with dendritic cells which could have been formed in situ (detected ex vivo in culture fluid) and/or *in vitro*, from mixtures of emigrated dendritic and T cells (46). As dendritic cell-T cell aggregates are regarded microenvironments in which immune activation and proliferation occur (47), it has been suggested that conjugation of these cells might contribute to cutaneous recall immune responses, such as the delayed type hypersensitivity in which memory T cells are engaged. Studying the cutaneous cell emigration and the corresponding cellular interactions may be of relevance in skin diseases with documented immunopathology. By using organ-cultured skin, the site of cutaneous viral HIV-1 selection was monitored and the virus was recovered from cells that had emigrated from skin explants (48).

Skin organ-culture systems enable studying mechanisms of cell emigration. A series of changes in the phenotype of dendritic cells, called "maturation", which include expression of molecules of the major histocompatibility complex (MHC) class II, and an array of costimulatory and adhesion molecules was demonstrated during their migration (45,49). These phenotypic changes represent the molecular basis of acquisition of strong stimulatory activity of dendritic cells detected in in vitro T-cell activation assays. Dendritic cells of the epidermis (Langerhans cells), appear to undergo these phenotypic changes within the epidermis during skin organ culture and these are accompanied by their spontaneous migration within the epidermis and then to the dermis (50). Phenotypic characteristics of DC could be modulated by cytokines of the epidermal origin (TNF-α and granulocyte-macrophage colony stimulating factor, GM-CSF) during organ culture, stressing a microenvironmental epidermal influence on the acquired migratory capacity.

Studies of the ear skin-sensitizing reaction using the mouse skin explant assay demonstrated the involvement of TNF-α and α_6/β_4 integrins (a key structural component of hemidesmosomes involved in the interaction between basal epidermal cells and the basement membrane) in the regulation of dendritic cell emigration from the epidermis (51). Migration from the epidermis through the dermis is characterized by the accumulation of dendritic cells in a characteristic string-like pattern, termed "cords", in the murine explant assay (44), and identified as lymphatic vessels in human skin organ cultures (52). Migratory dendritic cells, which accumulate in the dermal lymphatic vessels, are comprised of both epidermal and dermal dendritic cells as shown by ultrastructural and immunochemical criteria (49). Organ culture was employed in studies dealing with issues of epidermal dendritic cell emigration from the epidermis in response to skin-sensitizing chemicals, where it was shown that these cells are stimulated to migrate specifically in response to contact sensitizing agents, but not to nonsensitizing chemicals (49).

The skin organ culture model is also helpful for studying various biological aspects of wound healing in human skin including effects of growth factors (53) or transplanted keratinocytes (54), which might be of great value in better understanding the complex process that governs the healing of a human wound.

In conclusion, *in vitro* skin organ culture systems represent suitable and useful models to investigate various aspects of normal and abnormal skin biology (an overview into the use of skin organ culture is presented in Table 1). These systems may be used as principal or accompanying tests in situations where a confirmation of some clinical data is needed or their functional significance is investigated. They are particularly useful in studying effects of various pharmacological agents, and their possible mechanisms of action.

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