

## RESEARCH OUTPUTS / RÉSULTATS DE RECHERCHE

### The keratinocyte in cutaneous irritation and sensitization

Coquette, Alain; Berna, Nancy; Poumay, Yves; Pittelkow, Mark R.

*Published in:*

Biochemical modulation of skin reactions. Transdermals, topicals, cosmetics.

*Publication date:*

2000

*Document Version*

Publisher's PDF, also known as Version of record

[Link to publication](#)

*Citation for pulished version (HARVARD):*

Coquette, A, Berna, N, Poumay, Y & Pittelkow, MR 2000, The keratinocyte in cutaneous irritation and sensitization: Chapter 9. in F Agis, K John & J Wille (eds), *Biochemical modulation of skin reactions. Transdermals, topicals, cosmetics..* CRC Press, Boca Raton, pp. 125-143.

#### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

#### Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.



# 9 The Keratinocyte in Cutaneous Irritation and Sensitization

*Alain Coquette, Nancy Berna, Yves Poumay,  
and Mark R. Pittelkow*

## CONTENTS

I. Introduction .....	125
II. Keratinocyte Irritation or Sensitization: The Integrated Cell Response .....	127
III. Keratinocyte Elaborated Mediators .....	129
A. Interleukin 1 (IL-1) .....	129
B. Interleukin 6 (IL-6) .....	130
C. Interleukin 8 (IL-8) .....	130
D. Interleukin 10 (IL-10) .....	130
E. Interleukin 12 (IL-12) .....	131
F. Interleukin 15 (IL-15) .....	131
G. Tumor Necrosis Alpha (TNF- $\alpha$ ) .....	131
H. Chemokines — IP-10 etc. ....	131
I. Miscellaneous Mediators.....	131
IV. Models of Keratinocyte Irritancy and Sensitizer Testing.....	132
References .....	138

## I. INTRODUCTION

Epithelial tissues, including epidermis, tracheobronchial epithelium of lung, gastrointestinal epithelium, and uterine cervical epithelium, play a critical role in protecting man and other mammals from external environmental threats. Epithelial cells such as epidermal keratinocytes have long been known to provide a relatively impermeable barrier to outside factors that challenge the structural integrity and resilience of epidermis and other epithelia. However, only more recently have we discovered the active role played by the keratinocyte in initiating, modulating, and regulating responses of the skin as well as organism to the multitude of irritant or allergic (sensitizing) reactions that are part of daily existence. Keratinocytes express and, in some cases, secrete a plethora of biologically active molecules that mediate these responses. As the identification and biological function(s) of factors produced by keratinocytes continue to expand, the complexity and functional sophistication of epidermis become more apparent.

This chapter provides an overview and update on the role of the keratinocyte in cutaneous irritant and sensitization reactions. These findings significantly impact how skin reactions in dermal and transdermal delivery can be biochemically modulated. We also summarize various models that have been developed to better assess and predict epidermal irritation and sensitization. The cellular and molecular mechanisms mediating these responses in man will also be delineated.

The epidermis is a multilayered squamous epithelium that forms the interface between the organism and its environment. It is composed of several types of specialized resident or transient epithelial, neuroectodermal, and bone marrow-derived cells. These include epidermal keratinocytes to generate the protective barrier and provide for repair and regeneration of the epidermis, Langerhans cells, and T lymphocytes (T cells) for immunologic defense, melanocytes for pigment production and protection from ultraviolet radiation, and Merkel cells for neurocutaneous sensibility. Keratinocytes constitute the major cell type (>90%) and thus have the primary biologic role in providing both physical and biochemical attributes that maintain epidermal integrity and homeostasis. Epidermal keratinocytes also create a sentry function and compose the first level of communication with neighboring skin cells as well as other distant organs.<sup>1</sup>

The keratinocyte elaborates its protective function by undergoing a complex and finely coordinated program of cellular differentiation.<sup>2</sup> The basal layer consists of a single layer of proliferative and noncommitted keratinocytes, a fraction of which are functionally stem cells. The basal cell layer is anchored to the basal lamina via hemidesmosomes. These basal cells produce daughter cells that can either continue to populate the germinative layer or exit the basal layer to undergo terminal differentiation as they migrate to the epidermal surface. The spinous layer, constituting several or more cell layers, is located immediately above the basal layer and is characterized by the presence of extensive desmosomal connections between cells. The next morphologic layer, the granular layer, is distinguished by the presence of keratohyalin granules within the cytoplasm of the keratinocyte. Keratohyalin granules contain products of keratinocyte differentiation, such as loricrin, filaggrin, cystatin- $\alpha$ , and lipids that are used in the assembly of the corneocyte membrane and intercellular compartment. Another subcellular organelle, the keratinosome or lamellar body, is a specialized secretory vesicle present in the upper spinous and granular layers. Enzymes such as glucosylceramide synthase, lipid substrates/products such as glucocylceramides and sphingolipids, as well as specialized proteins such as corneodesmosin that make up the corneodesmosomes of the cornified layers, are also present in keratinosomes.<sup>3,4</sup> The transition zone delineates the region between nucleated and anucleate cells in upper epidermal layers. Within this region, selected cellular organelles and nucleic acids are targeted for elimination by the action of specific proteases, nucleases, and other enzymes. The final stage in keratinocyte terminal differentiation results in the formation of the cornified layer. This outermost layer is made up of corneocytes or "bricks" that form a packaged, stabilized array of keratin filaments, proteins, peptides, and other breakdown products contained within a cross-linked protein envelope and united by a lipid-rich intercellular "mortar."

Each stage of epidermal differentiation is characterized by specific biomarkers of gene expression. During normal epidermal differentiation, keratins 5 (K5) and 14 (K14) are expressed in the basal keratinocyte layer, while keratin 1 (K1) and 10 (K10) are expressed in the suprabasal layers. Involucrin is expressed in the late spinous layers and granular layers, and loricrin and filaggrin are specific markers of granular layers.

In the last decade, it has become clear that keratinocytes are not simply a mechanical barrier to the external environment, but are also able to produce a number of cytokines and other mediators with immunologic, inflammatory, and cell-adaptive (e.g., proliferative) properties. Cytokines are relatively small, soluble (glyco)proteins which are synthesized and secreted by various cells, bind to specific receptors, and regulate activation, proliferation, and differentiation of immune as well as nonimmune cells. They include several subclasses, designated: (1) interleukins (IL), (2) colony-stimulating factors (CSF), (3) interferons (IFN), (4) tumor necrosis factor (TNF) family members, (5) growth factors, and (6) suppressor factors.<sup>5,6</sup> Selected cytokines produced by keratinocytes in sensitization or irritation reactions will be reviewed here as well as in Chapter 12. We also will briefly review other keratinocyte-produced factors that mediate these responses. These include arachidonic acid and metabolites, biogenic amines, small molecular weight factors, and second-messenger molecules, as well as nitric oxide (NO) and reactive oxygen species (ROS). Together, these constitutive or inducible gene products and cellular metabolites of the keratinocyte directly or indirectly regulate the epidermal response to irritant or allergic agents contacting skin.

Figure 1 provides a schematic diagram of the events that induce the role of the keratinocyte in controlling inflammation within and between skin layers.

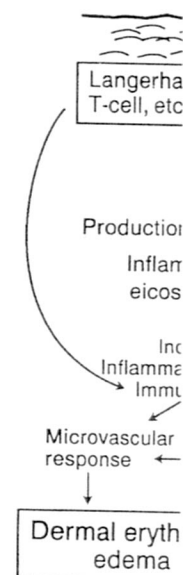


Figure 1 Sequence of events that induce the role of the keratinocyte in controlling inflammation within and between skin layers. (Adapted from Galli, *Toxicol. Lett.*)

Numerous proinflammatory cytokines that become produced by keratinocytes during these biologically mediated reactions are well characterized.

An important consideration in the pathogenesis of allergic reactions is the sensitization and sensitization of single cells and the subsequent epidermal reactions (e.g., to the allergen, histamine, etc.) In addition, the role of the keratinocyte as a "primary" cytokine-producing cell is insufficient to account for the response. However, sophisticated mathematical models have been developed to describe the biological "output" of these cells.

## II.

As depicted in Figure 1, the keratinocyte plays a central role in epidermal inflammation. In fact, it is the primary cell that is, some sensitization

surface between the resident or transient epidermal keratinocytes in the epidermis, Langerhans' cells, and melanocytes for pigment production. The role of keratinocytes in cutaneous responses is for pigment production, barrier homeostasis, and homeostasis. The role of keratinocytes at the first level of com-

and finely coordinated response of proliferative cells in the basal cell layer. Daughter cells that undergo terminal differentiation, migrating through several or more layers of the granular layer, and finally to the surface of the keratinocyte. Keratinocytes produce and secrete filaggrin, loricrin, filaggrin, and intercellular lipids, such as ceramides, as well as specific cornified layers, such as nucleated and non-nucleated cells and nucleic acids and other enzymes. The cornified layer is a stabilized array of keratin filaments within a cross-

linkage of gene expression. Keratinocytes are expressed in the suprabasal layers. Keratinocytes and filaggrin are

mechanical barrier and other mediators. Cytokines are produced by various cells, bind to receptors on immune as well as keratinocytes (IL), (2) colony-stimulating factors, family members, and other mediators in keratinocytes in response to injury. We also will discuss these include cytokines, and second-messengers (ROS). Together, keratinocytes directly respond to injury on skin.

Figure 1 provides a schematic framework depicting the sequence of cellular and biochemical events that induce irritant or sensitization reactions in epidermis. The keratinocyte plays a central role in controlling and coordinating cutaneous responses by other immune and inflammatory cells within and between the epidermis, dermis, and microvasculature.

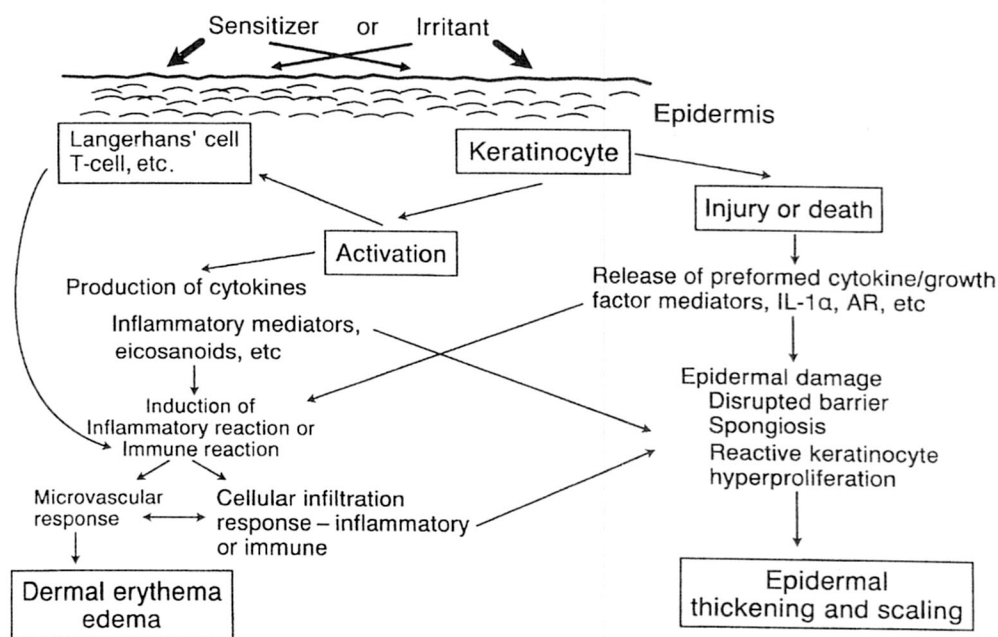


Figure 1 Sequence of events following irritant or sensitizer exposure to epidermis. (Modified from Corsini and Galli, *Toxicol. Lett.*, 102-103, 277-82, 1998.

Numerous protein and nonprotein factors are synthesized and secreted or released by keratinocytes that become "activated" by an irritant or allergen. A current, but inevitably incomplete list of these biologically active factors is presented in Table 1. The function(s) of some of these factors are well characterized while others are less well defined.

An important concept still to be comprehensively addressed for keratinocyte function in irritation and sensitization reactions is the hierarchy and ordering of events that take place within a single cell and the tissue to produce a given response. This concept is also critical for many other epidermal reactions to disease (e.g., psoriasis,<sup>7</sup> dermatitis, viral infections [verrucae-human papillomavirus, etc.]) In this context, some cytokines, such as IL-1 and TNF- $\alpha$ , have been considered to be "primary" cytokines, whereas others, such as IL-6, IL-8, and GM-CSF, are "secondary" since they are insufficient to induce an inflammatory response in the absence of other stimuli or primary cytokines.<sup>8</sup> However, the biological circuitry is no doubt much more complex and will likely require sophisticated mathematical modeling and application of neural network theory to fully describe the biological "output" of the keratinocyte that has been stimulated by an irritant or allergic "input."<sup>9</sup>

## II. KERATINOCYTE IRRITATION OR SENSITIZATION: THE INTEGRATED CELL RESPONSE

As depicted in Figure 1, irritants and allergens (haptens) have the ability to initiate similar responses in epidermis. In fact, irritants and sensitizers have the potential to overlap in their activity profiles; that is, some sensitizers also have irritant properties. The difference lies in the ability of a sensitizer

**TABLE 1**  
**Keratinocyte Mediators of Irritation and Sensitization**

	Cytokines
Primary	C-C chemokines
IL-1 $\alpha$	MCP-1
IL-1 $\beta$	MIP-1 $\alpha$
TNF- $\alpha$	RANTES
Humoral/cellular immune regulation	Growth factors
IL-10	TGF- $\alpha$
IL-12	AR
IL-18	HB-EGF
IFN- $\alpha$	NDF
IFN- $\beta$	VEGF
T cell growth	PDGF
IL-7	NGF
IL-15	FGFs
Colony-stimulating activity	Neurotrophin
IL-6	Suppressive/antagonist
G-CSF	IL-IRA
M-CSF	TGF- $\beta$
GM-CSF	IL-10
C-X-C chemokines	
IL-8	
Gro- $\alpha$ , - $\beta$ , - $\gamma$	
IP-10	
$\alpha$ -MSH	<b>Neuroendocrine</b>
	<b>Eicosenoids</b>
Arachidonate	12-HETE
PGE-2	LTB <sub>4</sub>
	<b>Oxygen-derived</b>
Nitric oxide (NO)	Superoxide (O <sub>2</sub> <sup>-</sup> )
Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	

to induce a specific immune response with immunological "memory." By contrast, cutaneous irritation is a nonimmunologic, reversible, local inflammatory reaction that induces edema and erythema following a single or repeated epicutaneous exposure to the chemical at a defined skin site.

Upon exposure of the keratinocyte to an irritant or sensitizer, cell injury or cell death (due to sufficiently severe damage induced by agents such as nitrogen or sulfa mustard agents<sup>10</sup>) occurs and triggers a set of responses in the keratinocyte and epidermis. Key to this response is IL-1 $\alpha$  release. Loss of barrier function by irritants, such as acetone, a strong delipidizing solvent, also can trigger rapid increase in expression of specific growth factors, such as amphiregulin (AR) and nerve growth factor (NGF).<sup>11</sup>

The keratinocyte becomes "activated" in response to irritant or sensitizer exposure. Specific sets of cytokines, as well as arachidonic acid metabolites and other inflammatory mediators, are expressed and secreted to trigger and modulate the inflammatory reaction. The ability of the keratinocyte to participate in generating effective signals for recruitment of Langerhans cells and T cells and propagating the afferent immune response places it within the central hub of the skin immune system (SIS).

The Keratinocyte in Cu

Whether induced erythema, edema, and and 10 delineate the response and allergic

In addition to expr keratinocyte also mod receptors, cell adhesio molecules likely play sensitization reactions and other cytokine fan cans, and numerous ot

Intracellular signal bled, and integrated int reactions in skin. The b cutaneous response. Fo the ability to induce sy ROS induced in human factor (EGF) receptor j naling pathways, includ (JNK), critical kinases c in part, terminate in the 1, AP-2,  $\gamma$ -interferon ac keratinocyte and many inhibitors of the inflam mones strongly inhibit / donic acid metabolizing

These findings link "activate" the keratinocy by UV or glucocorticoi zation (see also Chapter

In the following sec matory mediators produ This chapter also exam irritants and sensitizers

### III.

#### A. INTERLEUKIN 1 (IL-1)

IL-1 was originally des However, it is now well fibroblasts, and various 1 $\beta$ , encoded by distinct receptor types, suggestin as larger "pro-interleukin enzyme to the shorter bi necessary for its activity the predominant biologic lack constitutive IL-1 $\beta$  keratinocytes by both in be induced in epidermis

Whether induced by an irritant or a sensitizer, similar morphologic and histologic features of erythema, edema, and epidermal scaling and thickening (acanthosis) are observed. Chapters 6, 7, and 10 delineate the unique roles of the Langerhans cell and the T cell in the epidermal immune response and allergic contact dermatitis.

In addition to expressing and releasing potent cytokines and other inflammatory mediators, the keratinocyte also modulates expression of various immune and nonimmune related cell surface receptors, cell adhesion molecules, and extracellular matrix (ECM) factors. These cell-associated molecules likely play important roles in orchestrating the keratinocyte response during irritant and sensitization reactions. These gene products include ICAM-1, HLA-DR, receptors of growth factor and other cytokine families, integrins, cadherins, fibronectin, heparin sulfate and related proteoglycans, and numerous other cell-cell and ligand-receptor factors.

Intracellular signaling pathways of the keratinocyte are only beginning to be identified, assembled, and integrated into an intricate stimulus-response network that mediates irritant and sensitizer reactions in skin. The keratinocyte has the potential to either upregulate or downregulate a specific cutaneous response. For example, ultraviolet (UV) radiation induces cytokine cascades that have the ability to induce systemic immune suppression.<sup>12</sup> We have recently shown that H<sub>2</sub>O<sub>2</sub> and other ROS induced in human keratinocytes by UVB rapidly, but transiently, enhance epidermal growth factor (EGF) receptor phosphorylation and differentially activate downstream protein kinase signaling pathways, including extracellular regulated kinase (ERK), p38, and c-jun N-terminal kinase (JNK), critical kinases of mitogen- and stress-related cascades in keratinocytes.<sup>13,14</sup> These pathways, in part, terminate in the nucleus where specific transcription factors such as activator protein (AP)-1, AP-2,  $\gamma$ -interferon activation site (GAS), NF- $\kappa$ B, EGR, etc. regulate gene expression within the keratinocyte and many other cell types. In this regard, glucocorticoids are also known to be potent inhibitors of the inflammatory response. Recent studies have demonstrated that these steroid hormones strongly inhibit AP1, GAS, and NF- $\kappa$ B DNA-binding activities and induction of the arachidonic acid metabolizing enzyme, cyclooxygenase-2 (COX-2), in IL-1 $\beta$ -stimulated keratinocytes.<sup>15</sup>

These findings link cytokines and other inflammatory mediators to signaling pathways that "activate" the keratinocyte, but also demonstrate that keratinocyte responses can be downregulated by UV or glucocorticoids, well known and potent modulators of cutaneous irritation and sensitization (see also Chapters 15 and 20).

In the following sections, we provide a concise review of selected cytokines and other inflammatory mediators produced by keratinocytes that regulate cutaneous sensitization and irritancy. This chapter also examines the progress and comparative evaluation of *in vitro* models to test irritants and sensitizers using keratinocytes or more complex multicellular systems.

### III. KERATINOCYTE ELABORATED MEDIATORS

#### A. INTERLEUKIN 1 (IL-1)

IL-1 was originally described as a lymphocyte-activating factor produced only by monocytes. However, it is now well established that many cells, including epithelial cells, endothelial cells, fibroblasts, and various tumor cells, produce IL-1.<sup>16</sup> Two different forms of IL-1, IL-1 $\alpha$  and IL-1 $\beta$ , encoded by distinct genes, have been identified. These two forms bind to the same two IL-1 receptor types, suggesting they have similar biological activities. IL-1 $\alpha$  and IL-1 $\beta$  are synthesized as larger "pro-interleukins," which in the case of IL-1 $\beta$  must be cleaved by a specific converting enzyme to the shorter biologically active form. IL-1 $\alpha$  is also cleaved, but this does not seem to be necessary for its activity. Keratinocytes are able to synthesize and secrete both forms of IL-1, but the predominant biologically active form released by keratinocytes is IL-1 $\alpha$ ,<sup>17</sup> since keratinocytes lack constitutive IL-1 $\beta$  converting enzyme (ICE) activity. However, ICE activity is induced in keratinocytes by both irritant chemicals and sensitizers, such as urushiol.<sup>18</sup> IL-1 $\beta$  activation may be induced in epidermis *in vivo* by a non-ICE mechanism.<sup>19</sup> This contrasts with observations in

contrast, cutaneous induces edema and at a defined skin site. or cell death (due to ard agents<sup>10</sup>) occurs s response is IL-1 $\alpha$  dizing solvent, also hiregulin (AR) and

exposure. Specific tory mediators, are The ability of the ngerhans cells and ral hub of the skin

in vitro suggesting lack of IL-1 $\beta$  processing.<sup>20</sup> IL-1 $\alpha$  appears to be retained intracellularly or in a membrane-bound form. As long as the epidermis is intact, IL-1 is eliminated by normal desquamation. Because IL-1 lacks a hydrophobic leader sequence necessary for transmembrane secretion, it has been proposed that it only can be released after some type of cell injury or membrane perturbation.<sup>16</sup> In human skin, the levels of IL-1 are 100 to 1000 times higher than in most other tissues. Keratinocytes are able to produce it constitutively without stimulation. Upregulation of IL-1 synthesis has been observed upon stimulation with lipopolysaccharides (LPS), phorbol myristate acetate (PMA), physical, chemical, or thermal injury, ultraviolet irradiation, and a variety of cytokines (i.e., GM-CSF, TNF- $\alpha$ , IL-6, TGF- $\alpha$ , and IL-1 $\alpha$  and IL-1 $\beta$  itself).<sup>21</sup> Interestingly, IL-1 $\beta$  appears to be specifically induced by hapten within 1 to 3 h of exposure, whereas IL-1 $\alpha$  mRNA is not induced by either hapten or primary irritants, as measured by reverse transcriptase-polymerase chain reaction (RT-PCR).<sup>22</sup> Furthermore, IL-1 $\beta$  induces Langerhans cell migration out of epidermis and neutralizing antibody to IL-1 $\beta$ , but not IL-1 $\alpha$ , TNF- $\alpha$ , or GM-CSF, prevented allergen-induced migration of Langerhans cells, suggesting that IL-1 $\beta$  plays a role in irritation of contact hypersensitivity.<sup>23</sup> The effects of IL-1 are highly pleiotropic and space limits delineation of all of its biological effects. For further detailed information on IL-1, see Chapter 12.

IL-1 is a proinflammatory cytokine. It is chemotactic for monocytes, lymphocytes, and neutrophils. It stimulates the proliferation, differentiation, and activation of various cells and the production of other cytokines such as GM-CSF, IL-6, and IL-8. Keratinocytes, in addition to producing IL-1, express large amounts of specific IL-1 receptors and IL-1 receptor antagonists (IL-1ra).<sup>24</sup> This antagonist binds to the same receptor as IL-1, but it does not produce cell activation and so acts as a competitive inhibitor to prevent IL-1 effects unless IL-1 exceeds certain threshold levels. The reader is referred to Chapter 14 for further information on IL-1 and IL-1ra effects.

#### B. INTERLEUKIN 6 (IL-6)

IL-6 is a multifunctional cytokine released by many different cells, including monocytes, fibroblasts, endothelial cells, keratinocytes, and different tumor cells.<sup>25</sup> Unstimulated keratinocytes usually produce low levels of IL-6, but expression can be upregulated by the addition of stimulants such as IL-1, LPS, PMA, or UV-B irradiation, TNF- $\alpha$ , GM-CSF, IL-4, TGF- $\beta$ , and injury.<sup>6</sup> Like IL-1, IL-6 has a variety of biological activities on different target cells. Many biological effects of IL-1 and IL-6 overlap. IL-6 may augment proliferation of keratinocytes. Moreover, some evidence suggests that IL-6 plays a role as mediator in inflammatory skin diseases such as psoriasis.<sup>21</sup> Compared to other cytokines and growth factors, the potency of IL-6 in these responses is less pronounced and likely secondary.

#### C. INTERLEUKIN 8 (IL-8)

In addition to monocytes, a variety of cells including endothelial cells, keratinocytes, fibroblasts, and T lymphocytes produce IL-8.<sup>26</sup> Keratinocytes do not produce IL-8 constitutively, but the production is stimulated by other cytokines (IL-1 $\alpha$ , IL-1 $\beta$ , TNF- $\alpha$ , and IFN- $\gamma$ ), LPS, and phorbol esters.<sup>27</sup> IL-8 is strongly chemotactic for polymorphonuclear neutrophils and lymphocytes, increases cytosolic free calcium, and induces granule exocytosis.<sup>28</sup> IL-8 is also chemotactic for human basophils and stimulates them to release histamine.<sup>27</sup> Therefore, IL-8 is also classified as a potent chemokine of the C-X-C class.<sup>26</sup>

#### D. INTERLEUKIN 10 (IL-10)

Originally described as a product of bone marrow-derived cells, IL-10 is also produced by activated murine keratinocytes.<sup>29</sup> IL-10 is known to be an anti-inflammatory cytokine and may act as a suppressor factor of immune reactions. IL-10 expression is enhanced in UV-treated keratinocytes, and hapten-specific tolerance induced by UVB is mediated by IL-10.<sup>30</sup> It may inhibit the production

The Keratinocyte in

of cytokines such as TNF- $\alpha$  and IL-1 $\beta$  promotes induction of skin by reducing the

#### E. INTERLEUKIN 12 (IL-12)

IL-12 is a heterodimeric cytokine that plays a role in sensitization responses and is induced to effect by IL-12 strongly stimulates

#### F. INTERLEUKIN 15 (IL-15)

IL-15 has recently been shown to be a cytokine. IL-15 is known to have a function to IL-2, and it is a proinflammatory agent that downregulates

#### G. TUMOR NECROSIS FACTOR- $\alpha$ (TNF- $\alpha$ )

TNF- $\alpha$  is a pleiotropic cytokine including proliferation and TNF- $\alpha$  also induces cell death as well as inducing ICAM-1 in skin.<sup>34</sup> Selected protein in epidermis

#### H. CHEMOKINES - MIP-1

Chemokines such as MIP-1 (MCP)-1 have been shown to be chemotactic and a chemokine in keratinocytes in the epidermal skin. Neutrophilic allergic and

#### I. MISCELLANEOUS

Products of the arachidonic acid pathway, such as prostaglandins and polyunsaturated fatty acids, are potent activators of phospholipases (A2) and cyclooxygenase (COX) and themselves activate.<sup>37,38</sup> A variety of skin which may be

The keratinocyte response to UV radiation, etc. We have shown that in keratinocytes fol

of cytokines such as IFN- $\gamma$ , IL-1, and TNF- $\alpha$ . By inhibiting IFN- $\gamma$  production by Th1 cells, it promotes induction of a Th2 response. One role of IL-10 may be to prevent severe damage to the skin by reducing the risk of necrosis by an ongoing inflammatory process.

#### E. INTERLEUKIN 12 (IL-12)

IL-12 is a heterodimeric protein and a potent costimulator of Th1 cells that are involved in cutaneous sensitization responses. Keratinocytes constitutively express the lower Mr (35 kDa) chain of IL-12 and are induced to express the 40-kDa chain following exposure to contact allergen, but not irritants.<sup>31</sup> IL-12 strongly stimulates T cell proliferation and mediates the primary immune response in skin.

#### F. INTERLEUKIN 15 (IL-15)

IL-15 has recently been shown to be induced in epidermal keratinocytes by culture and selected cytokines. IL-15 is a potent immunomodulator of T cell-mediated immune responses, similar in function to IL-2, and attracts and activates antigen-specific Th1 cells. IL-15 also stimulates the proinflammatory and antimicrobial properties of neutrophils. Both UVB exposure and corticosteroids downregulate IL-15 expression in keratinocytes.<sup>32</sup>

#### G. TUMOR NECROSIS ALPHA (TNF- $\alpha$ )

TNF- $\alpha$  is a pleiotropic proinflammatory cytokine that mediates a range of biological responses, including proliferation, apoptosis, and inducing gene responses in TNF receptor-bearing cells. TNF- $\alpha$  also induces inflammation in skin following local synthesis and release or by injection as well as inducing ICAM-1 expression in keratinocytes.<sup>33</sup> Irritants such as SDS and PMA also have been shown to rapidly induce TNF- $\alpha$  expression as well as subsequent inflammation and edema in skin.<sup>34</sup> Selected allergens such as nickel and DNFB also induce TNF- $\alpha$  gene expression and protein in epidermis of sensitized animals.<sup>35</sup>

#### H. CHEMOKINES — IP-10 ETC.

Chemokines such as interferon-induced protein (IP)-10 and macrophage chemotactic protein (MCP)-1 have been shown to be upregulated in cutaneous delayed-type hypersensitivity reactions and other epidermal responses. Chemokines play an important role in inflammation via T cell chemotactic and adhesion-promoting activities. Interferon- $\gamma$  strongly stimulates expression of IP-10 in keratinocytes.<sup>36</sup> IP-10 and other selected chemokines expressed by keratinocytes function in the epidermal signaling network to localize and induce specific responses that mediate cutaneous allergic and irritant reactions.

#### I. MISCELLANEOUS MEDIATORS

Products of the arachidonic acid metabolic pathway (termed "eicosenoids"), as well as arachidonate itself, are potent regulators of inflammation and allergic or irritant epidermal responses. The polyunsaturated fatty acid precursor, arachidonic acid, is produced by the enzymatic action of phospholipases (A<sub>2</sub> or C) on lipids of the cell membrane. In addition to the well-known actions of the cyclooxygenase, lipoxygenase, and monooxygenase metabolites of arachidonate in skin, arachidonic acid itself has been shown to trigger keratinocyte stress-activated responses, such as JNK activation.<sup>37,38</sup> A variety of the early events in skin inflammation are mediated by arachidonic acid and its metabolites. Tumor promoters and other irritants induce arachidonic acid metabolism in skin which may be used as relevant markers for cutaneous irritation.<sup>39,40</sup>

The keratinocyte also generates various free radicals following stimulation by chemical agents, UV radiation, etc. We have recently shown that superoxide and H<sub>2</sub>O<sub>2</sub> are rapidly produced and eliminated in keratinocytes following exposure to UVB<sup>13,14</sup> and other agents. These ROS potentially regulate levels



These mediators may be present in the epidermis.

## IN VITRO TESTING

and environmental factors, such as cosmetics, pharmaceuticals, and chemicals, can lead to skin irritation. The goal of in vitro testing is to identify compounds that cause skin irritation before they are used in human products. Various animal models, such as the guinea pig maximization test, mouse ear swelling test, and human skin equivalents, are used to assess skin irritation. However, these models have limitations, such as variability and ethical concerns. In vitro testing offers a more controlled and ethical alternative. It involves testing compounds on cultured skin cells or reconstructed skin models. This approach allows for the study of skin irritation mechanisms at the cellular level and can help identify potential irritants before they reach the market. In vitro testing is becoming increasingly important in the cosmetics and pharmaceutical industries due to its ability to provide detailed insights into skin irritation pathways and to reduce the need for animal testing.

In vitro alternatives to animal testing, such as skin equivalents, offer a more controlled and ethical approach to studying skin irritation. These models are typically limited by their inability to fully replicate the complex structure and function of human skin.

Human skin equivalents, such as collagen gels and fibroblast-populated collagen matrices, are used to study skin irritation. However, these models often lack the full complement of skin cells and structures found in vivo. Skin equivalents derived from human skin, such as those from SkinEthic and Organogenesis, are composed of differentiated epithelium and

cornified epidermis with significantly improved barrier function and metabolic activity.<sup>57,66-68</sup> The presence of a stratum corneum makes it possible to apply topically a wide variety of products and/or complex formulations. Differentiation markers such as suprabasal keratins, integrin  $\beta 4$ , integrin  $\alpha 6$ , fibronectin, involucrin, filaggrin, trichohyalin, type I, III, IV, V, and VII collagen, laminin, heparan sulfate, and membrane-bound transglutaminase have been found to be expressed similar to those of the epidermis.<sup>58,69-71</sup> Moreover, keratin synthesis and the production of cornified envelopes parallels that found in vivo. Spinous cells display abundant glycogen deposit, and keratohyalin granules are more abundant in the granular layer. Both the size and number of hemidesmosomes increase during maturation in vitro and anchoring fibrils are observed.<sup>58,66,69,72-74</sup>

Percutaneous penetration studies performed with human skin recombinant models have revealed that the stratum corneum forms a substantial barrier to  $^3\text{H}$ -water,<sup>53,63</sup> pindolol, calcitonin,<sup>75</sup> toluene, carbazole, benzopyrene,<sup>63</sup> testosterone,<sup>55,76,77</sup> estradiol,<sup>55</sup> hydrocortisone,<sup>55,76,78</sup> benzoic acid,<sup>15,77</sup> cyclosporine,<sup>79</sup> salicylic acid, provitamin B5, theophylline, and scopolamine.<sup>77</sup> The results obtained are more consistent and reproducible than cadaver skin<sup>79</sup> and correlate well with those recorded for hairless guinea pig skin.<sup>75</sup> Nevertheless, the relative permeability of normal human skin compared to reconstructed skin is different and is likely to vary considerably from one compound to another. A good correlation for one class of chemicals is not necessarily indicative of a similar relationship for other chemicals.<sup>53,55,63,77</sup> This points to an impaired barrier function of reconstructed epidermis in vitro. In fact, despite the similarity in tissue architecture, reconstructed epidermis exhibits some deviations from normal epidermis, depending on the tissue culture method and the source of keratinocytes. Reduction of ceramides 4 to 7 and 6 to 7, integrin overexpression, premature expression of specific differentiation markers, and abnormal lipid composition have been observed under normal in vitro culture conditions.<sup>71,80,81</sup> By using freeze-fracture electron microscopy,<sup>82</sup> X-ray diffraction,<sup>83,84</sup> and confocal laser scanning microscopy,<sup>85</sup> it has been shown that, in some cases, reconstructed epidermis displays abnormalities in lamellar body delivery and extrusion, which manifests itself by a disturbance of the transformation of lamellar bodies into lamellar lipid bilayers by impaired structural organization and distribution of epidermal lipids into the intercellular space.<sup>86,87</sup> Furthermore, by using small-angle X-ray diffraction techniques, it has been shown that the stratum corneum lipids appear to be organized in multilamellar structures with a periodicity of 12 nm<sup>87</sup> in contrast to native epidermis, in which two lamellar phases with periodicities of 6.4 and 13.4 nm are typically detected.<sup>83</sup> Consequently, whereas for native epidermis the penetration pathway is confined only to the extracellular space, diffusion within the stratum corneum in the reconstructed epidermis likely occurs via both extracellular and intracellular pathways.<sup>85</sup> These findings may partially explain the divergent results obtained from various percutaneous penetration studies.

Improvements in the culture conditions, such as maintaining the cultures in delipidized serum, reduction of the relative humidity,<sup>53</sup> and use of chemically defined medium,<sup>57,88</sup> has led to further optimization of these models. Epidermal tissues generated at 33°C in absence of epidermal growth factor,<sup>52,71</sup> and in the presence of vitamin C<sup>71</sup> but absence of retinoic acid,<sup>57,88</sup> improves the stratum corneum architecture and lipid profile. In vitamin C-supplemental medium, the content of glucosylceramides and of ceramides 6 and 7 is markedly increased.<sup>71</sup> In absence of serum, the relative amounts of ceramides, free fatty acids, and cholesterol are comparable to native epidermis.<sup>52</sup> Epidermis reconstructed on fibroblast-populated collagen at 37°C in the presence of EGF has a similar morphology to that of native epidermis. However, irrespective of the culture conditions, involucrin is aberrantly expressed. EGF supplementation has a deleterious effect on epidermal morphogenesis and differentiation. The synthesis of K1 and K10 is suppressed on both protein and mRNA levels.<sup>71</sup>

Since 1990, a fully differentiated epithelium having the features of in vivo epidermis has been obtained in vitro by culturing second-passage normal human keratinocytes in a retinoic acid-free, chemically defined medium MCDB 153 on inert filter substrates exposed to the air-liquid interface for 14 days.<sup>57</sup> In this model, the basal cells synthesize and secrete all major markers of hemidesmosomes as well as components of the lamina lucida. Hemidesmosomes with major dense plaques and anchoring filaments and a basement membrane-like structure were identified, suggesting that

the presence of serum and dermal factors is not required.<sup>58</sup> Because of the restricted presence of exogenous growth factors and protein in the medium, this *in vitro* human living epidermis is approaching the most suitable system for detecting and testing the effects of any product that has the potential to be in contact with epidermis.<sup>58,89</sup>

An advantage of *in vitro*-reconstructed skin equivalents is the possibility of incorporating various additional cell types alone or in combination with keratinocytes. Recently, the introduction of melanocytes into epidermal reconstructs has expanded potential applications of these models.<sup>90</sup> As in the *in vivo* state, melanocytes appear as dendritic cells and are located in the basal keratinocyte layer. Melanin has been detected in both the melanocytes and the neighboring keratinocytes. Following UV radiation, increase in the number of dopa-positive melanocytes in the basal layer has been shown that results in increased pigmentation of the irradiated skin equivalent. More recent advances in culture techniques have made it possible to develop reconstructed epidermis containing not only keratinocytes but melanocytes and Langerhans cells as well. Cord blood-derived CD34+ hematopoietic progenitor cells induced to differentiate by GM-CSF and TNF- $\alpha$  were seeded onto a reconstructed epidermis composed of keratinocytes and melanocytes. This culture system gives rise to a reconstructed *in vitro* model displaying a pigmented epidermis with melanocytes in the basal layer and resident epidermal Langerhans cells located suprabasally and expressing major histocompatibility complex class II, CD1 antigen, and Birbeck granules.<sup>91</sup> It provides an attractive *in vitro* system to study the regulation of melanogenesis and melanocyte-keratinocyte interactions, and to investigate in a more defined model how these processes are affected by UV irradiation. In addition, this epidermal model can be used to test the phototoxic or photoprotective potential of various compounds as well as sunscreens, which is a distinct advantage over other animal models.

*In vitro* reconstructed epidermis allows testing of products at concentrations and in formulations that would be used *in vivo*. In addition, the dose-response relationship can be examined over a wide range of concentrations. Furthermore, the lower part of the tissue is bathed in the culture medium that can be withdrawn for analysis of released mediators. They provide quantifiable and objective end point measurements compared to those *in vivo* studies where more subjective parameters, such as erythema and edema, are often used. For these reasons, reconstructed human epidermis can be widely exploited for various research purposes, including studies of cutaneous biogenesis and skin wound healing, investigation of the regulation of keratinocyte differentiation, pharmaceutical agent metabolism studies and absorption properties,<sup>63,79,92-95</sup> assessment of cutaneous immunotoxicological response,<sup>61</sup> and responses to irritants<sup>56,58,62,65,69,96-98</sup> and to sensitizers.<sup>99,100</sup>

The end points most frequently used include histological analysis of tissue damage, cell membrane damage estimated by measuring leakage of enzymes such as lactate dehydrogenase (LDH);<sup>56</sup> cell viability determination by MTT conversion<sup>62,65,89,96,101,102</sup> or Neutral Red assay;<sup>101</sup> the modulation of the stratum barrier function and the release of proinflammatory mediators, such as IL-1 $\alpha$ ,<sup>56,61,62,89,96,103,104</sup> IL-1 $\beta$ , and IL-6,<sup>56,103</sup> IL-8,<sup>56</sup> TNF- $\alpha$ ,<sup>61</sup> prostaglandins;<sup>56,62,96,105,106</sup> hydroxyeicosanetraeno (HETEs) and leukotriene B<sub>4</sub> (LTB<sub>4</sub>);<sup>107</sup> plasminogen activator;<sup>96</sup> cytokine mRNA expression;<sup>108-111</sup> antileukoproteinase synthesis;<sup>112</sup> ICAM-1 expression;<sup>81</sup> integrin receptor modulation;<sup>81</sup> measure of intracellular ATP<sup>113</sup> and corneorosometry.<sup>114</sup>

Upon reaching the living layers of the epidermis, irritant and sensitizing agents modulate cell membrane integrity. Irritation *in vivo* modulates integrin expression.<sup>81</sup> Keratinocytes in the basal layer of healthy epidermis express four different integrins, namely,  $\alpha$ 2 $\beta$ 1,  $\alpha$ 3 $\beta$ 1,  $\alpha$ 6 $\beta$ 4, and  $\alpha$ 5 $\beta$ 5; they participate in keratinocyte adhesion to the basement membrane that separates the epidermis from the dermis. Integrins have been shown to be involved in keratinocyte differentiation and activation, cell-cell adhesion between keratinocytes, and keratinocyte migration on extracellular matrix proteins.<sup>115</sup> Under inflammatory conditions, upregulation and suprabasal expression of these integrins coupled with the induction of  $\alpha$ 5 $\beta$ 1 and intercellular adhesion molecule-1 (ICAM-1), a specific ligand for  $\beta$ 2 integrins, have been demonstrated.<sup>116</sup> Finally, in skin reconstructed *in vitro*, UVB exposure leads to major epidermal developmental changes characterized by a downregulation of major markers of keratinocyte differentiation such as keratin 10, loricrin, filaggrin, and keratinocyte transglutaminase (Type I).<sup>117</sup>

Irritants and sensitizers modulate the ability of keratinocytes to elaborate and release cytokines at elevated levels. These cytokines, including IL-1, IL-6, IL-8, and IL-15, are members of specific monokine families. Consequently, the expression of these cytokines has been proposed as a reliable marker of skin irritation. The complexity of the skin response to irritants and sensitizers has led to the development of a possible approach to the study of the cytokine mRNA expression in the skin as one possible approach.

Cultured keratinocytes produce a variety of cytokines, including IL-1, IL-6, IL-8, and IL-15. These cytokines have been shown to be involved in chemotaxis and activation of T cells.<sup>29,121</sup> Cultured keratinocytes produce IL-1 $\alpha$ , -1 $\beta$ , -6, and -1 $\beta$  and keratinocyte-derived IL-8.

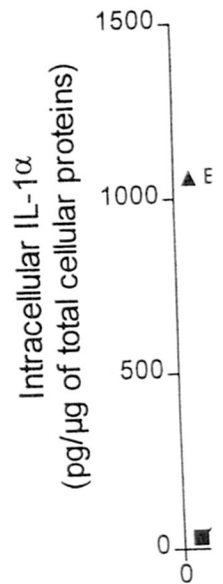
Studies have shown that irritants and immunomodulators such as IL-15, GM-CSF, and TNF- $\alpha$  modulate the expression of IL-1 $\alpha$ , -1 $\beta$ , -6, -8, and -1 $\beta$ . For IL-6 and IL-8 are experiments performed with sodium lauryl sulfate showed an increase in IL-1 $\alpha$  mRNA expression. The message was expressed for IL-1 $\alpha$ . The molecular mechanism of the ability of irritants and sensitizers to modulate cytokine production and determine the differentiation of keratinocytes from a resting state to a stimulated state by benzalkonium chloride. Both irritants and allergens modulate the levels for IL-1 $\beta$  in the skin. The allergens produce an increase in the levels of IL-6 and IL-8.

For the SKIN2<sup>TM</sup> model, three contact allergens modulate the message levels for IL-1 $\alpha$ , -1 $\beta$ , -6, -8, and -1 $\beta$ . The state level of IL-15 in the epidermal cytokines is modulated. Frequently, it should be used in investigating the differentiation of keratinocytes. In this respect, the modulation of keratinocytes and sensitizers by autocrine regulation of dendritic cells in lymphocytes and Langerhans cells.<sup>127</sup> The expression and release of cytokines by neutrophils and T lymphocytes.



Recently, a model of reconstructed human epidermis (RHE) was used as an *in vitro* skin model to discriminate the effects of Tween 80, Triton X100, and benzalkonium chloride (BC) as irritants and 1-chloro-2,4-dinitrobenzene (DNCB) as a sensitizing agent.<sup>131</sup> It is based on the model developed by Rosdy and Cross<sup>59</sup> and consists of a mitotically and metabolically active culture of human-derived epidermal keratinocytes that are differentiated into basal, spinous, granular, and cornified layers analogous to those found *in vivo*.<sup>58</sup> Specific markers of epidermal differentiation such as keratins 1/10, involucrin, filagrin, loricrin, and transglutaminase have been localized. The lipid profile analysis shows that this model contains free fatty acids and all classes of ceramides. These cultures exhibit barrier function and metabolic activity which allow direct application of the product to be tested, thus simulating *in vivo* human topical exposure and an *in vivo* skin irritation/sensitization test.<sup>58,89</sup> In the experiment, the extent of epidermal irritation and sensitization was evaluated morphologically and amounts of intracellular and extracellular of IL-1 $\alpha$  and IL-8 were assayed. The corresponding constitutive mRNA levels of these interleukins were quantified and the cytotoxic response was assessed by a MTT assay. The RHE resembled normal human epidermis with all typical epidermal layers. Keratin 10 was typically confined to the suprabasal layers of the tissue, suggesting normal epidermal terminal differentiation. Topical application of each of the three surfactants resulted in significant changes of tissue morphology and was coupled with different dose-dependent decreases in cell viability corresponding to their *in vivo* irritant potency.<sup>119,130,131</sup> IL-1 $\alpha$  release was shown to increase inversely with decrease in cell viability, but interestingly, the surfactants did not stimulate increase in IL-8 levels. In contrast, DNCB did not induce elevated IL-1 $\alpha$  release, although it induced a rapid dose-dependent decrease in cell viability. By contrast, DNCB increased IL-8 release. RT-PCR demonstrated the presence of mRNA for IL-1 $\alpha$  and for IL-8 as previously described *in vivo*.<sup>132,133</sup> IL-1 $\alpha$  was the most abundant cytokine transcript. BC, Triton X100, and DNCB upregulated IL-8 mRNA expression, while only BC induced a significant increase in IL-1 $\alpha$  mRNA expression. The results demonstrate that the production of IL-1 $\alpha$  and its release into the extracellular medium were due not only to specific cytotoxicity, but also to the extent of direct epidermal tissue stimulation. Conversely, the production of IL-8 did not directly correlate with cytotoxicity but may be linked to the type of product applied and classified as either irritant or sensitizer. These findings emphasize the requirement to use substances of the same class as standard controls in order to test unknown compounds that will be coupled with the investigation of multiple end points. Our data demonstrate that divergence of the IL-1 $\alpha$  and IL-8 releases profiles and corresponding mRNA upregulation differentiates between specific responses to irritants or allergens. These findings suggest that it may be possible in a single integrated assay to classify and discriminate between irritant and sensitizing agents as a function of patterns of induced cytokine production and cell viability measurements. It has not been determined which mechanism is responsible for the change in cytokine mRNA expression, but we have observed that mRNA levels do not necessarily correlate with protein expression, and we also find that the type of product appears to determine the pattern of cell mediator expression and release. This could explain the disparate results obtained with the EpiDerm or the Skin2 models where only mRNA expression was investigated.<sup>100</sup> Our results suggest that skin allergens and skin irritants could stimulate variable patterns of epidermal cytokine production in RHE. The stimulation seems to be nonspecific in terms of mRNA signal strength, but specific in terms of protein production and release. In fact, if the cytokine levels (intracellular vs. extracellular) are plotted, a strong correlation for IL-1 $\alpha$  ( $R = 0.999$ ) is observed, suggesting a direct relationship among synthesis, storage, and release. By contrast, we observe for IL-8 that BC and Triton X100 induces synthesis and storage without significant release, while DNCB induces a rapid synthesis and release of IL-8 without storage (Figure 2). These observations highlight the complexity of biochemical pathways underlying cytokine production, and suggest interactions with different specific cellular target sites.

Functional mitochondria seem to be required in keratinocytes for *de novo* IL-1 $\alpha$  synthesis.<sup>134</sup> In fact, tributyltin, a well-known skin irritant in rodent and human, causes disturbance in the



**Figure 2** Correlation between intracellular IL-1 $\alpha$  and cell viability after topical application of Tween 80, Triton X100, and benzalkonium chloride (BC).

respiratory chain of mitochondria, specifically the ubiquinone site which acts as a mobile electron carrier. In *in vitro* experiments, the RHE treated with tributyltin, as demonstrated by decreased cell viability, showed a decrease in IL-1 $\alpha$  release observed with the control. This suggests that mitochondrial dysfunction may be involved in the RHE cytokine release. In addition, humoral factors such as TNF- $\alpha$  and IL-1 $\beta$  are known to be involved in the RHE cytokine release, producing reactive oxygen species *in vivo*.<sup>136</sup> In addition, humoral factors such as TNF- $\alpha$  and IL-1 $\beta$  are known to be regulated by protein kinases.

In conclusion, the results demonstrate that the reconstructed human epidermis (RHE) model is useful in terms of their biochemical and morphological characteristics. The merged ones and than analyzed the results. The results as well as solid materials can be used for predicting the irritant and sensitizing potential of a product. The cytokine secretion profile that it is a complex array of mRNA upregulation, protein synthesis, and release. The combination of cell viability, mRNA upregulation, protein synthesis, and release variations, irrespective of the same or different dose, can be used as a useful *in vitro* model for predicting the irritant and sensitizing potential of a number of products in the presence of different chemicals. The possibility of using specific patterns of inflammatory cytokine release as alternatives to animal tests.

in vitro skin model  
 icide (BC) as irritants  
 based on the model  
 ally active culture of  
 inous, granular, and  
 ermal differentiation  
 e been localized. The  
 classes of ceramides.  
 direct application of  
 and an in vivo skin  
 tion and sensitization  
 r of IL-1 $\alpha$  and IL-8  
 kins were quantified  
 bled normal human  
 ed to the suprabasal  
 opical application of  
 gy and was coupled  
 heir in vivo irritant  
 in cell viability, but  
 rast, DNCB did not  
 ease in cell viability.  
 ce of mRNA for IL-  
 abundant cytokine  
 on, while only BC  
 emonstrate that the  
 not only to specific  
 sely, the production  
 e of product applied  
 requirement to use  
 ompounds that will  
 e that divergence of  
 ferentiates between  
 ay be possible in a  
 sitizing agents as a  
 rements. It has not  
 mRNA expression,  
 ein expression, and  
 mediator expression  
 Derm or the Skin2  
 that skin allergens  
 ction in RHE. The  
 pecific in terms of  
 . extracellular) are  
 direct relationship  
 C and Triton X100  
 s a rapid synthesis  
 the complexity of  
 ons with different  
 IL-1 $\alpha$  synthesis.<sup>134</sup>  
 disturbance in the

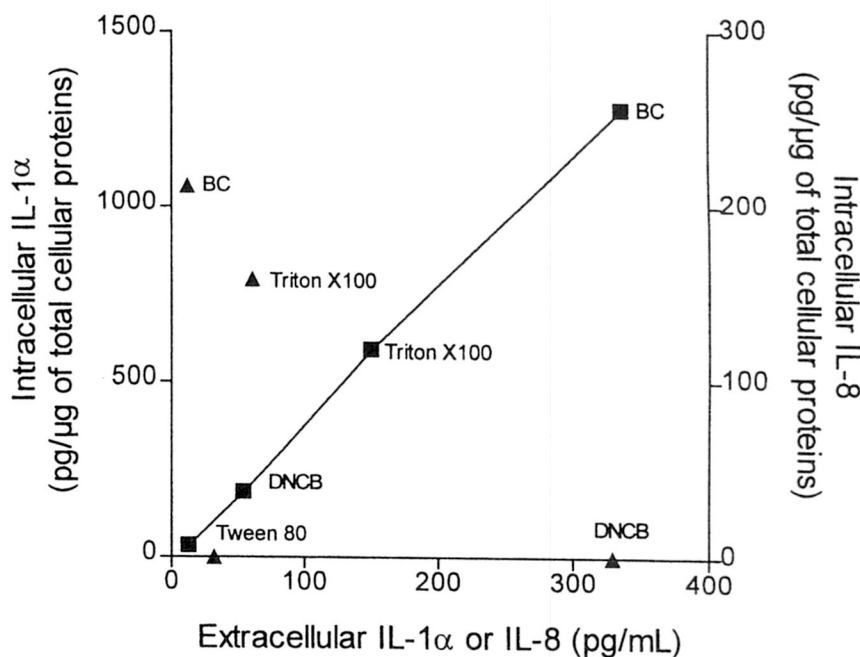


Figure 2 Correlation between the intra- and extracellular levels of IL-1 $\alpha$  (■) and IL-8 (▲) in the RHE after topical application of Tween 80, Triton X100, BC, and DNCB (20 h, 37°C, 5%CO<sub>2</sub>).

respiratory chain of mitochondria, probably by production of reactive oxygen intermediates at the ubiquinone site which activates transcription factor and promotes IL-1 $\alpha$  synthesis.<sup>134,135</sup> In our experiments, the RHE treatments with Triton X100, BC, and DNCB reduce mitochondrial function as demonstrated by decreased MTT conversion and could partially explain the results. However, the release observed with DNCB suggests that mechanisms other than mitochondrial activity may be involved in the RHE cytokine production. In fact, DNCB increases NADPH oxidase enzymatic activity, producing reactive oxygen intermediates that mediate effects of this hapten on cells in vivo.<sup>136</sup> In addition, human keratinocyte IL-8 synthesis may be either positively or negatively regulated by protein kinase C depending on the stimulus.<sup>137</sup>

In conclusion, the reconstructed human epidermal equivalents more closely resemble native tissue in terms of their biosynthetic, morphological, and barrier properties than conventional submerged ones and than animal skins do. Due to the presence of the stratum corneum, water-insoluble as well as solid materials can be applied topically and are better suited than conventional cultures for predicting the irritation and sensitization potentials of topically applied agents. Divergent cytokine secretion profiles characterize the RHE response to irritants and sensitizers, suggesting that it is a complex array of signals that determines the type of protein released, not only in terms of mRNA upregulation, but above all in terms of interaction with the signal transduction. The combination of cell viability measurement with the quantification of IL-1 $\alpha$  and IL-8 allows the classification and discrimination between irritant and sensitizing agents. The low interexperimental variations, irrespective of whether the experiments are performed on RHE derived from cells of the same or different donors, indicate that the RHE grown in defined medium represent a very useful in vitro model for toxicological studies which correlates with in vivo results. However, the number of products is not actually sufficient to extend the correlation across different classes of chemicals. The possibility that other irritant or sensitizing agents from different classes may exhibit specific patterns of inflammatory mediators would provide for the validation of in vitro models as alternatives to animal testing.

## REFERENCES

1. Pittelkow, M. R., *Principles and Practice of Endocrinology and Metabolism*, 2nd ed., Becker, K. L., Ed., Lippincott, Williams and Wilkins, Philadelphia, PA, 1995, 1526.
2. Eckert, R. L., Structure, function, and differentiation of the keratinocyte, *Physiol. Rev.*, 69, 1316, 1989.
3. Watanabe, R., Wu, K., Paul, P., Marks, D. L., Kobayashi, T., Pittelkow, M. R., and Pagano, R. E., Up-regulation of glucosylceramide synthase expression and activity during human keratinocyte differentiation, *J. Biol. Chem.*, 273, 9651, 1998.
4. Guerrin, M., Simon, M., Montezin, M., Haftek, M., Vincent, C., and Serre, G., Expression of cloning of human corneodesmosin proves its identity with the product of the S gene and allows improved characterization of its processing during keratinocyte differentiation, *J. Biol. Chem.*, 273, 22640, 1998.
5. Sauder, D. N., The role of epidermal cytokines in inflammatory skin diseases, *J. Invest. Dermatol.*, 95, 27S, 1990.
6. Luger, T. A. and Schwartz, T., *Epidermal Growth Factors and Cytokines*, 1st ed., Luger, T. A. and Schwartz, T., Eds., Marcel Dekker, New York, 1994.
7. Pittelkow, M. R., *Psoriasis*, 3rd ed., Roenigk, H. H., Jr. and Maibach, H. I., Eds., Marcel Dekker, New York, 1998, 225.
8. Kupper, T. S., The activated keratinocyte: a model for inducible cytokine production by non-bone marrow-derived cells in cutaneous inflammatory and immune responses, *J. Invest. Dermatol.*, 94, 146S, 1990.
9. Bhella, U. S. and Iyengar, R., Emergent properties of networks of biological signaling pathways, *Science*, 283, 381, 1999.
10. Smith, K. J., Smith, W. J., Hamilton, T., Skelton, H. G., Graham, J. S., Okerberg, C., Moeller, R., and Hackley, B. E., Histopathologic and immunohistochemical features in human skin after exposure to nitrogen and sulfur mustard, *Am. J. Dermatopathol.*, 20, 22, 1998.
11. Liou, A., Eliase, P. M., Granfeld, C., Feingold, K. R., and Wood, L. C., Amphiregulin and nerve growth factor expression are regulated by barrier status in murine epidermis, *J. Invest. Dermatol.*, 108, 73, 1997.
12. Shreedhar, V., Giese, T., Sung, V. W., and E, U. S., A cytokine cascade including prostaglandin E2, IL-4, and IL-10 is responsible for UV-induced systemic immune suppression, *J. Immunol.*, 160, 3783, 1998.
13. Peus, D., Vasa, R. A., Meves, A., Pott, M., Beyerle, A., Squillace, K., and Pittelkow, M. R., H<sub>2</sub>O<sub>2</sub> is an important mediator of UVB-induced EGF-receptor phosphorylation in cultured keratinocytes, *J. Invest. Dermatol.*, 110, 966, 1998.
14. Peus, D., Vasa, R. A., Beyerle, A., Meves, A., Krautmacher, C., and Pittelkow, M. R., UVB activates ERK1/2 and p38 signaling pathways via reactive oxygen species in cultured keratinocytes, *J. Invest. Dermatol.*, 112, 751, 1999.
15. Lukiw, W. J., Pelaez, R. P., Martinez, J., and Bazan, N. G., Budesonide epimer R or dexamethasone selectively inhibit platelet-activating factor-induced or interleukin 1beta-induced DNA binding activity of cis-acting transcription factors and cyclooxygenase-2 gene expression in human epidermal keratinocytes, *Proc. Natl. Acad. Sci. U.S.A.*, 95, 3914, 1998.
16. Dinarello, C. A., Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist, *Int. Rev. Immunol.*, 16, 457, 1998.
17. Kupper, T. S. and Groves, R. W., The interleukin-1 axis and cutaneous inflammation, *J. Invest. Dermatol.*, 105, 62S, 1995.
18. Zepter, K., Haffner, A., Soohoo, L. F., DeLuca, D., Tang, H. P., Fisher, P., Chavinson, J., and Elmet, C. A., Induction of biologically active IL-1 beta-converting enzyme and mature IL-1 beta in human keratinocytes by inflammatory and immunologic stimuli, *J. Immunol.*, 159, 6203, 1997.
19. Nylander-Lundqvist, E., Back, O., and Egelrud, T., IL-1 beta activation in human epidermis, *J. Immunol.*, 15, 1699, 1996.
20. Mizutani, H., Black, R., and Kupper, T. S., Human keratinocytes produce but do not process pro-interleukin-1 (IL-1) beta. Different strategies of IL-1 production and processing in monocytes and keratinocytes, *J. Clin. Invest.*, 87, 1066, 1991.
21. Ansel, J., Cytokine modulation of keratinocyte cytokines, *J. Invest. Dermatol.*, 94, 101S, 1990.
22. Matsunga, T., Kataya induced by hapten di, *J. Dermatol.*, 25, 421
23. Rambukkana, A., Pis allergens on human I molecules, and early
24. Phillips, W. G., Feldr network in keratinocy 101, 177, 1995.
25. Akira, S., Taga, T., a 1993.
26. Proost, P., Wuyts, A., Res., 26, 211, 1996.
27. Wilmer, J. L. and Lu in human epidermal I 11, 37, 1995.
28. Barker, J. N. W. N., M as initiators of inflar
29. Enk, A. H. and Katz, Acad. Sci. U.S.A., 89
30. Niizeki, H. and Strei radiation of skin is n
31. Muller, G., Saloga, J. Identification and inc
32. Blauvelt, A., Asada, is expressed by hun downregulated by ul
33. Kondo, S. and Saude for TNF-alpha-induc
34. Lisby, S., Muller, K. regulate tumor necro mechanisms, *Int. Im*
35. Little, M. C., Gawk normal and transforr prevention of Cr(VI)
36. Boorsma, D. M., Fli C. P., and Stoof, T. J Res., 190, 335, 1998
37. Pentland, A. P., Fitz Eisen, A. Z., Wolff, McGraw-Hill, New
38. Meves, A., Peus, D., and lipid peroxidatic Dermatol., 112, 533
39. Muller-Decker, K., I Arachidonic acid me with surfactants, *To*
40. Li-Stiles, B., Lo, H. by tumor promoters
41. Draize, J. H., Wood substances applied t 1994.
42. Magnusson, B. and guinea pig maximiz
43. Buehler, E. V., Dela

22. Matsunga, T., Katayama, I., Yokozeki, H., and Nishioka, K., Epidermal cytokine mRNA expression induced by hapten differs from that induced by primary irritant in human skin organ culture system, *J. Dermatol.*, 25, 421, 1998.
23. Rambukkana, A., Pistoro, F. H., Bos, J. D., Kapsenberg, M. L., and Das, P. K., Effects of contact allergens on human Langerhans' cells in skin organ culture: migration, modulation of cell surface molecules, and early expression of interleukin-1 beta protein, *Lab. Invest.*, 74, 422, 1996.
24. Phillips, W. G., Feldmann, M., Breathnach, S. M., and Brennan, F. M., Modulation of the IL-1 cytokine network in keratinocytes by intracellular IL-1 alpha and IL-1 receptor antagonist, *Clin. Exp. Immunol.*, 101, 177, 1995.
25. Akira, S., Taga, T., and Kishimoto, T., Interleukin-6 in biology and medicine, *Adv. Immunol.*, 54, 1, 1993.
26. Proost, P., Wuyts, A., and van Damme, J., The role of chemokines in inflammation, *Int. J. Clin. Lab. Res.*, 26, 211, 1996.
27. Wilmer, J. L. and Luster, M. I., Chemical induction of interleukin-8, a proinflammatory chemokine, in human epidermal keratinocyte cultures and its relation to cytogenetic toxicity, *Cell. Biol. Toxicol.*, 11, 37, 1995.
28. Barker, J. N. W. N., Mitra, R. S., Griffiths, C. E. M., Dixit, V. M., and Nickoloff, B. J., Keratinocytes as initiators of inflammation, *Lancet*, 337, 211, 1991.
29. Enk, A. H. and Katz, S. I., Early molecular events in the induction of contact sensitivity, *Proc. Natl. Acad. Sci. U.S.A.*, 89, 1398, 1992a.
30. Niizeki, H. and Streilein, J. W., Hapten-specific tolerance induced by acute, low-dose ultraviolet B radiation of skin is mediated via interleukin-10, *J. Invest. Dermatol.*, 109, 25, 1997.
31. Muller, G., Saloga, J., Germann, T., Bellinghausen, I., Mohamadzadeh, M., Knop, J., and Enk, A. H., Identification and induction of human keratinocyte-derived IL-12, *J. Clin. Invest.*, 94, 1799, 1994.
32. Blauvelt, A., Asada, H., Klaus-Kovtun, V., J. A. D., Lucey, D. R., and I, K. S., Interleukin-15 mRNA is expressed by human keratinocytes Langerhans cells, and blood-derived dendritic cells and is downregulated by ultraviolet B radiation, *J. Invest. Dermatol.*, 106, 1047, 1996.
33. Kondo, S. and Sauder, D. N., Tumor necrosis factor (TNF) receptor type 1 (p55) is a main mediator for TNF-alpha-induced skin inflammation, *Eur. J. Immunol.*, 27, 1713, 1997.
34. Lisby, S., Muller, K. M., Jongeneel, C. V., Saurat, J. H., and Hauser, C., Nickel and skin irritants up-regulate tumor necrosis factor-alpha mRNA in keratinocytes by different but potentially synergistic mechanisms, *Int. Immunol.*, 7, 343, 1995.
35. Little, M. C., Gawkrödger, D. J., and MacNiel, S., Chromium- and nickel-induced cytotoxicity in normal and transformed human keratinocytes: an investigation of pharmacological approaches to the prevention of Cr(VI)-induced cytotoxicity, *Br. J. Dermatol.*, 134, 199, 1996.
36. Boorsma, D. M., Flier, J., Sampat, S., Ottevanger, C., de Haan, P., Hooft, L., Willemze, R., Tensen, C. P., and Stoof, T. J., Chemokine IP-10 expression in cultured human keratinocytes, *Arch. Dermatol. Res.*, 190, 335, 1998.
37. Pentland, A. P., *Fitzpatrick's Dermatology in General Medicine*, Vol. 1, 5th ed., Freedberg, I. M., Eisen, A. Z., Wolff, K., Austen, K. F., Goldsmith, L. A., Katz, S. I., and Fitzpatrick, T. B., Eds., McGraw-Hill, New York, 1999, 432.
38. Meves, A., Peus, D., and Pittelkow, M. R., Arachidonic acid-induced generation of superoxide anion and lipid peroxidation mediates c-jun-N-terminal kinase activation in cultured keratinocytes, *J. Invest. Dermatol.*, 112, 533, 1999.
39. Muller-Decker, K., Heinzelmann, T., Furstenberger, G., Kecskes, A., Lehmann, W. D., and Marks, F., Arachidonic acid metabolism in primary irritant dermatitis produced by patch testing of human skin with surfactants, *Toxicol. Appl. Pharmacol.*, 153, 159, 1998.
40. Li-Stiles, B., Lo, H. H., and Fischer, S. M., Differential activation of keratinocyte phospholipase A2S by tumor promoters and other irritants, *Adv. Exp. Med. Biol.*, 407, 117, 1997.
41. Draize, J. H., Woodard, G., and Calvery, H. O., Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes, *J. Pharmacol. Exp. Ther.*, 82, 377, 1944.
42. Magnusson, B. and Kligman, A. M., The identification of contact allergens by animal assay. The guinea pig maximization test, *J. Invest. Dermatol.*, 52, 268, 1969.
43. Buehler, E. V., Delayed contact hypersensitivity in the guinea pig, *Arch. Dermatol.*, 91, 171, 1965.

44. Kimber, I. and Weisenberger, C., A murine local lymph node assay for the identification of contact allergens. Assay development and results of an initial validation study, *Arch. Toxicol.*, 63, 274, 1989.
45. Gad, S. C., Dunn, B. J., Dobbs, D. W., Reilly, C., and Walsh, R. D., Development and validation of an alternative dermal sensitization test: the mouse ear swelling test (MEST), *Toxicol. Appl. Pharmacol.*, 84, 93, 1986.
46. Nixon, G. A., Tyson, C. A., and Wertz, W. D., Interspecies comparison of skin irritancy, *Toxicol. Appl. Pharmacol.*, 31, 481, 1975.
47. Riviere, J. E., Sage, B. H., and Monterio, N. A., Transdermal lidocaine iontophoresis in isolated perfuse porcine skin, *J. Toxicol. Cut. Ocular. Toxicol.*, 8, 493, 1990.
48. Ahmed, S., Imai, T., and Otagiri, M., Stereoselectivity in cutaneous hydrolysis and transdermal transport of propranolol prodrug, *Enantiomer*, 2, 181, 1997.
49. De Leo, V. A., Harber, L. C., Kong, B. M., and S.J., D. S., Surfactant-induced alteration of arachidonic acid metabolism of mammalian cells in culture, *Proc. Soc. Exp. Biol. Med.*, 184, 477, 1987.
50. Brosin, A., Wolf, W., Mattheus, A., and Heise, H., Use of XTT-assay to assess the cytotoxicity of different surfactants and metal salts in human keratinocytes (HACAT) — A feasible method for in vitro testing of skin irritants, *Acta. Dermatol. Venereol.*, 77, 26, 1997.
51. Holbrook, K. A. and Hennings, H., Phenotype expression of epidermal cell in vitro: a review, *J. Invest Dermatol.*, 81, 11S, 1983.
52. Gibbs, S., Vicanova, J., Bouwstra, J., Valstar, D., Kempenaar, J., and Ponec, M., Culture of reconstructed epidermis in a defined medium at 33 degrees C shows a delayed epidermal maturation, prolonged lifespan and improved stratum corneum, *Arch. Dermatol. Res.*, 289, 585, 1997.
53. Regnier, M., Caron, D., Reichert, U., and Schaeffer, H., Reconstructed human epidermis: a model to study in vitro the barrier function of the skin, *Skin Pharmacol.*, 5, 42, 1992.
54. Augustin, C., Collombel, C., and Damour, O., Measurements of the protective effect of topically applied sunscreens using in vitro three-dimensional dermal and skin equivalents, *Photochem. Photobiol.*, 66, 853, 1997.
55. Ernesti, A. M., Swiderk, M., and Gay, R., Absorption and metabolism of topically applied testosterone in organotypic skin culture, *Skin Pharmacol.*, 5, 146, 1992.
56. Ponec, M. and Kempenaar, J., Use of human skin recombinants as an in vitro model for testing the irritation potential of cutaneous irritants, *Skin Pharmacol.*, 8, 49, 1995.
57. Rosdy, M. and Clauss, L. C., Terminal epidermal differentiation of human keratinocytes grown in chemically defined medium on inert filter substrates at the air-liquid interface, *J. Invest. Dermatol.*, 95, 409, 1990.
58. Rosdy, M., Pisani, A., and Ortonne, J. P., Production of basement membrane components by a reconstructed epidermis cultured in the absence of serum and dermal factors, *Br. J. Dermatol.*, 129, 227, 1993.
59. Prunieras, M., Schweizer, J., Michel, S., Bailly, C., and Prunieras, M., Methods for cultivation of keratinocytes with an air-liquid interface, *J. Invest. Dermatol.*, 81, 28S, 1983.
60. Harriger, M. D. and Hull, B. E., Cornification and basement membrane formation in a bilayered human skin equivalent maintained at an air-liquid interface, *J. Burn Care Rehabil.*, 13, 187, 1992.
61. Rheins, L. A., Edwards, S. M., Mia, O., and Donnelly, T. A., Skin 2TM: an in vitro model to assess cutaneous immunotoxicity, *Toxicol. In Vitro*, 8, 1007, 1994.
62. Gay, R. S. M., Nelson, D., and Ernesti, A., The living skin equivalent as a model in vitro for ranking the toxic potential of dermal irritants, *Toxicol. In Vitro*, 6, 303, 1992.
63. Yang, J. J. and Krueger, A., Evaluation of two commercial human skin cultures for in vitro percutaneous absorption, *In Vitro Toxicol.*, 5, 211, 1992.
64. Slivka, S. R., Testosterone metabolism in an in vitro skin model, *Cell. Biol. Toxicol.*, 8, 267, 1992.
65. De Wever, B. and Rheins, L. A., Skin 2TM: an in vitro skin analog, *Alt. Meth. Toxicol.*, 10, 121, 1994.
66. Parnigotto, P. P., Bernuzzo, S., Bruno, P., Conconi, M. T., and Montesi, F., Characterization and applications of human epidermis reconstructed in vitro in de-epidermized derma, *Farmaco*, 53, 125, 1998.
67. Slivka, S. R., Laudeen, L. L., Zimber, M. P., and Bartel, R. L., Biochemical characterization barrier function and drug metabolism in an in vitro skin model, *J. Cell Biol.*, 115, 2072, 1991.
68. Geesin, J. C., Brown, L. J., Liu, Z. Y., and Berg, R. A., Development of a skin model based on insoluble fibrillar collagen, *J. Biomed. Mater. Res.*, 33, 1, 1996.
69. Fleischmajer, R., MacF fibroblast co-culture m
70. Rosdy, M., Fartasch, M tuted human epidermi
71. Ponec, M., Gibbs, S., growth factor and tem 1997.
72. Vicanova, J., Mommae in the in vitro reconst
73. Stoppie, P., Borghraef an in vitro reconstru 1300/2000), *Eur. J. M*
74. van de Sandt, J. J., Bc tiation in skin organ cu *Anim.*, 31, 761, 1995.
75. Hager, D. F., Mancuso TM as a model memb
76. Regnier, M., Caron, D reconstructed epiderm
77. Doucet, O., Garcia, N. human skin for assess
78. Godwin, D. A., Michn skin alternative, *J. Phu*
79. Vickers, A. E. M., Big A and SDZ IMM 125 57, 215, 1995.
80. Nickoloff, B. J. and N. cytokine cascade in hu
81. von den Driesch, P., F ICAM-1 on keratinocy sulphate, *Arch. Derma*
82. Bodde, H. E., Holmar Freeze-fracture electro 1990.
83. Bouwstra, J. A., Goori stratum corneum by si
84. Bouwstra, J. A., Goori of human stratum corn study, *Int. J. Pharmac*
85. Simonetti, O., Hoogstr and Ponec, M., Visuali reconstructed epiderm
86. Fartasch, M. and Pone culture systems, *J. Inv*
87. Ponec, M., Bouwstra, K. R., James, V. J., an
88. Rosdy, M., Fartasch, M of human epidermis re
89. Doucet, O., Robert, C pharmacological mode
90. Todd, C., Hewitt, S. D.. and keratinocytes at th
91. Regnier, M., Staquet, pigmented reconstruct

69. Fleischmajer, R., MacDonald, E. D., Contard, P., and Perlish, J. S., Immunocytochemistry of a keratinocyte-fibroblast co-culture model for reconstruction of human skin, *J. Histochem. Cytochem.*, 41, 1359, 1993.
70. Rosdy, M., Fartasch, M., and Darmon, M., Normal permeability barrier to tritiated water in reconstituted human epidermis, 1997.
71. Ponc, M., Gibbs, S., Weerheim, A., Kempenaar, J., Mulder, A., and Mommaas, A. M., Epidermal growth factor and temperature regulate keratinocyte differentiation, *Arch. Dermatol. Res.*, 289, 317, 1997.
72. Vicanova, J., Mommaas, A. M., Mulder, A. A., Koerten, H. K., and Ponc, M., Impaired desquamation in the in vitro reconstructed human epidermis, *Cell Tissue Res.*, 186, 115, 1996.
73. Stoppie, P., Borghraef, P., De Wever, B., Geysen, J., and Borgers, M., The epidermal architecture of an in vitro reconstructed human skin equivalent (Advanced Tissue Sciences Skin2 Models ZK 1300/2000), *Eur. J. Morphol.*, 31, 26, 1993.
74. van de Sandt, J. J., Bos, T. A., and Rutten, A. A., Epidermal cell proliferation and terminal differentiation in skin organ culture after topical exposure to sodium dodecyl sulphate, *In Vitro Cell Dev. Biol. Anim.*, 31, 761, 1995.
75. Hager, D. F., Mancuso, F. A., Nazareno, J. P., Sharkey, J. W., and Siverly, J. R., Evaluation of Testskin TM as a model membrane, *Proc. Int. Symp. Control. Rel. Bioact. Mater.*, 19, 487, 1992.
76. Regnier, M., Caron, D., Reichert, U., and Schaeffer, H., Barrier function of human skin and human reconstructed epidermis, *J. Pharm. Sci.*, 82, 404, 1993.
77. Doucet, O., Garcia, N., and Zastrow, L., Skin culture model: a possible alternative to the use of excised human skin for assessing in vitro percutaneous absorption, *Toxicol. In Vitro*, 12, 423, 1998.
78. Godwin, D. A., Michniak, B. B., and Creek, K. E., Evaluation of transdermal enhancers using a novel skin alternative, *J. Pharmaceutical. Sci.*, 86, 1001, 1997.
79. Vickers, A. E. M., Biggi, W. A., Dannecker, R., and Fisher, V., Uptake and metabolism of cyclosporin A and SDZ IMM 125 in the human in vitro Skin 2TM dermal and barrier function models, *Life Sci.*, 57, 215, 1995.
80. Nickoloff, B. J. and Naidu, Y., Perturbation of epidermal barrier function correlates with initiation of cytokine cascade in human skin, *J. Am. Acad. Dermatol.*, 30, 535, 1994.
81. von den Driesch, P., Fartasch, M., Huner, A., and Ponc, M., Expression of integrin receptors and ICAM-1 on keratinocytes in vivo and in an in vitro reconstructed epidermis: effects of sodium dodecyl sulphate, *Arch. Dermatol. Res.*, 287, 249, 1995.
82. Bodde, H. E., Holman, B., Spies, F., Weerheim, A., Kempenaar, J., Mommaas, M., and Ponc, M., Freeze-fracture electron microscopy on in vitro reconstructed epidermis, *J. Invest. Dermatol.*, 95, 108, 1990.
83. Bouwstra, J. A., Gooris, G. S., van der Spek, J. A., and Bras, W., Structural investigations of human stratum corneum by small-angle x-ray scattering, *J. Invest. Dermatol.*, 97, 1007, 1991.
84. Bouwstra, J. A., Gooris, G. S., Salomon-de Vries, M. A., van der Spek, J. A., and Bras, W., Structure of human stratum corneum as a function of temperature and hydration: A wide-angle x-ray diffraction study, *Int. J. Pharmaceutics*, 84, 205, 1991.
85. Simonetti, O., Hoogstraate, A. J., Bialik, W., Kempenaar, J. A., Schrijvers, A. H. G. J., Bodde, H. E., and Ponc, M., Visualization of diffusion pathways across the stratum corneum of native and in vitro reconstructed epidermis by confocal laser scanning microscopy, *Arch. Dermatol. Res.*, 1995.
86. Fartasch, M. and Ponc, M., Improved barrier structure formation in air-exposed human keratinocyte culture systems, *J. Invest. Dermatol.*, 102, 366, 1994.
87. Ponc, M., Bouwstra, J., and Fartasch, M., *Prediction of Percutaneous Penetration*, Vol. 3b, Brain, K. R., James, V. J., and Walters, K. A., Eds., STS Publishing, Cardiff, U.K., 1993, 428.
88. Rosdy, M., Fartasch, M., and Ponc, M., Structurally and biochemically normal permeability barrier of human epidermis reconstituted in chemically defined medium, *J. Invest. Dermatol.*, 107, 664, 1996.
89. Doucet, O., Robert, C., and Zastrow, L., Use of a serum-free reconstituted epidermis as a skin pharmacological model, *Toxicol. In Vitro*, 10, 305, 1996.
90. Todd, C., Hewitt, S. D., Kempenaar, J., Noz, K., Thody, A. J., and Ponc, M., Co-culture of melanocytes and keratinocytes at the air-liquide interface, *Arch. Dermatol. Res.*, 285, 455, 1993.
91. Regnier, M., Staquet, M. J., Schmitt, D., and Schmidt, R., Integration of Langerhans' cells into a pigmented reconstructed human epidermis, *J. Invest. Dermatol.*, 109, 510, 1997.

92. Boyce, S., Michel, S., Reichert, U., Schroot, B., and Schmidt, R., Reconstructed skin from cultured human keratinocytes and fibroblasts on a collagen-glycosaminoglycan biopolymer substrate, *Skin Pharmacol.*, 3, 136, 1990.
93. Ponc, M., Wauben-Penris, P. J., Burger, A., Kempenaar, J., and Bodde, H. E., Nitroglycerin and sucrose permeability as quality markers for reconstructed human epidermis, *Skin Pharmacol.*, 3, 126, 1990.
94. Lapiere, C. M. and Nusgens, B. V., The various uses of in-vitro reconstituted skin, *Bull. Mem. Acad. R. Med. Belg.*, 145, 235, 1990.
95. Magnaldo, T., Bernerd, F., Asslineau, D., and Darmon, M., Expression of loricrin is negatively controlled by retinoic acid in human epidermis reconstructed in vitro, *Differentiation*, 49, 39, 1992.
96. Slivka, S. R. and Zeigler, F., Use of an in vitro skin model for determining epidermal and dermal contributions to irritant response, *J. Toxicol. Cut. Ocular Toxicol.*, 12, 49, 1993.
97. Nickoloff, B. J., The cytokine network in psoriasis, *Arch. Dermatol.*, 127, 871, 1991.
98. Kubilus, J., Cannon, C., and Neal, P., Response of the EpiDerm skin model to topically applied irritants and allergens, *Toxicol. In Vitro*, 9, 157, 1996.
99. Sikorski, E. E., Gerberick, G. F., and Limardi, L. C., Evaluation of cytokine message levels in the EpiDerm TM in vitro skin model following application of contact allergens and skin irritants, *J. Invest. Dermatol.*, 108, 662, 1997.
100. Sikorski, E. E., Gerberick, G. F., and Limardi, L. C., Evaluation of a human skin equivalent model as a potential in vitro system to predict the contact sensitization potential of chemicals, 106, 939, 1996.
101. Triglia, D., Braa, S. S., Yonan, C., and Naughton, G. K., Cytotoxicity testing using neutral red and MTT assays on a three-dimensional human skin substrate, *Toxicol. In Vitro*, 5, 573, 1991.
102. Edwards, S. M., Donnelly, T. A., Sayre, R. M., and Rheins, L. A., *European Medicines Research*, Fracchia, G. N., Ed., IOS Press, 1994, 106.
103. Arlian, L. G., Vyszenskimoher, D. L., Rapp, C. M., and Hull, B. E., Production of IL-1-alpha and IL-1-beta by human skin equivalents parasitized by *Sarcoptes scabiei*, *J. Parasitol.*, 82, 719, 1996.
104. Noelhudson, M. S., Brautboucher, F., Robert, M., Aubrey, M., and Wepierre, J., Comparison of six different methods to assess UVA cytotoxicity on reconstructed epidermis-relevance of a fluorimetric assay (the calcein-am) to evaluate the photoprotective effects of alpha-tocopherol, *Toxicol. In Vitro*, 11, 645, 1997.
105. Roguet, R., Dossou, K. G., and Rougier, A., *In Vitro Skin Toxicology: Irritation, Phototoxicity, Sensitization*, Rougier, A., Goldberg, A. M., and Maibach, H. I., Eds., Mary Ann Liebert, New York, 1994, 141.
106. Sato, T., Kirimura, Y., and Mori, Y., The co-culture of dermal fibroblasts with human epidermal keratinocytes induces increased prostaglandin E2 production and cyclooxygenase 2 activity in fibroblasts, *J. Invest. Dermatol.*, 109, 334, 1997.
107. Dykes, P. J., Edwards, M. J., Donovan, M. R., Merrett, V., Morgan, H. E., and Marks, R., In vitro reconstruction of human skin: The use of skin equivalents as potential indicators of cutaneous toxicity, *Toxicol. In Vitro*, 5, 1, 1991.
108. Corsini, E., Terzoli, A., Bruccoleri, A., Marinovich, M., and Galli, C. L., Induction of tumor necrosis factor-alpha in vivo by a skin irritant, tributyltin, through activation of transcription factors: pharmacological modulation by anti-inflammatory drugs, *J. Invest. Dermatol.*, 108, 892, 1997.
109. Burleson, F. G., Limardi, L. C., Sikorski, E. E., Rheins, L. A., Donnelly, T. A., and Gerberick, G. F., Cytokine mRNA expression in an in vitro human skin model SKIN2(TM), *Toxicol. In Vitro*, 10, 511, 1996.
110. Boelsma, E., Tanojo, H., Bodde, H. E., and Ponc, M., Assessment of the potential irritancy of oleic acid on human skin-evaluation in vitro and in vivo, *Toxicol. In Vitro*, 10, 729ff, 1996.
111. Boelsma, E., Tanojo, H., Bodde, H. E., and Ponc, M., An in vitro study of the use of a human skin equivalent for irritancy screening of fatty acids, *Toxicol. In Vitro*, 11, 365, 1997.
112. Boelsma, E., Gibbs, S., and Ponc, M., Expression of skin-derived antileukoproteinase (SKALP) in reconstructed human epidermis and its value as a marker for skin irritation, *Acta. Derm. Venereol.*, 78, 107, 1998.
113. Buche, P., Violin, L., and Girard, P., Evaluation of the effects of cosmetic or dermo-pharmaceutical products on cutaneous energy metabolism using the Episkin model of reconstructed epidermis, *Cell Biol. Toxicol.*, 10, 381, 1994.
114. Goffin, V., Paye, M., and others, by anionic surfactants
115. Watt, F. M. and Jones, Suppl. 1, 185, 1993.
116. Kellner, I., Konter, U., 6) on psoriatic keratin
117. Bernerd, F. and Assel, morphogenesis after sy
118. Enk, A. H. and Katz, 149, 92, 1992b.
119. Roguet, R. D., K.G., et al, a preliminary study, *J.*
120. Dickson, F. M., Lawrence, keratinocyte cultures fr
121. Barker, J. N. W. N., et al, 1992.
122. Knop, J. and Enk, A., sensitivity, *Int. Arch. A*
123. Holliday, M. R., Dearn, of cutaneous interleuki
124. Limardi, L. C., Sikorski, EpiDerm cultures, *J. I*
125. Konstantinove, N., Fri, cultures and is further i
126. Waelti, E. R., Inaebit, S, dermal fibroblast feeder 805, 1992.
127. Kupper, T., Interleukin characterization, *Adv. D*
128. Cumberbatch, M. and K, to draining lymph node nology, 75, 257, 1992.
129. Gueniche, A., Viac, J., haptens on intracellular keratinocytes, *Eur. J. De*
130. Bettley, F. R., The toxic
131. Singer, E. J. and Pitts, E
132. Rowbottom, A. W., Nort lymphoid organs in hum
133. Anttila, H. S. I., Reitan immunoreactivity in the psoriasis, *J. Invest. Derr*
134. Corsini, E., Shubert, C., i interleukin-1-alpha produ
135. Corsini, E., Bruccoleri, associated with skin irrit
136. Arner, E. S. J., Bjornste inhibitor of human thioir epanied by a large increas
137. Chabot-Fletcher, M., Bre regulated by protein kina

114. Goffin, V., Paye, M., and Pierard, G. E., Comparison of in vitro predictive tests for irritation induced by anionic surfactants, *Contact Dermatitis*, 33, 38, 1995.
115. Watt, F. M. and Jones, P. H., Expression and function of the keratinocytes integrins, *Development*, Suppl. 1, 185, 1993.
116. Kellner, I., Konter, U., and Sterry, W., Overexpression of extracellular matrix receptors (VLA-3,5 and 6) on psoriatic keratinocytes, *Br. J. Dermatol.*, 123, 211, 1991.
117. Bernerd, F. and Asselineau, D., Successive alteration and recovery of epidermal differentiation and morphogenesis after specific UVB damages in skin reconstructed in vitro, *Dev. Biol.*, 183, 123, 1997.
118. Enk, A. H. and Katz, S. I., Identification and induction of keratinocyte-derived IL-10, *J. Immunol.*, 149, 92, 1992b.
119. Roguet, R. D., K.G., and Rougier, A., Use of in vitro skin recombinants to evaluate cutaneous toxicity: a preliminary study, *J. Toxicol. Cut Ocular Toxicol.*, 11, 305, 1994.
120. Dickson, F. M., Lawrence, J. N., and Benford, D. J., Release of inflammatory mediators in human keratinocyte cultures following exposure to a skin irritant, *Toxicol. In Vitro*, 7, 385, 1993.
121. Barker, J. N. W. N., Role of keratinocytes in allergic contact dermatitis, *Contact Dermatitis*, 26, 145, 1992.
122. Knop, J. and Enk, A. H., Cellular and molecular mechanisms in the induction phase of contact sensitivity, *Int. Arch. Allergy Immunol.*, 107, 231, 1995.
123. Holliday, M. R., Dearman, R. J., Corsini, E., Basketter, D. A., and Kimber, I., Selective stimulation of cutaneous interleukin 6 expression by skin allergens, *J. Appl. Toxicol.*, 16, 65, 1996.
124. Limardi, L. C., Sikorski, E. E., and Gerberick, G. F., Cytokine gene expression in the MatTek EpiDerem cultures, *J. Invest. Dermatol.*, 106, 929, 1996.
125. Konstantinove, N., Friant, S., and Hazarika, P., IL-8 release is highly elevated in skin equivalent cultures and is further induced by psoriatic patient fibroblasts, *J. Invest. Dermatol.*, 104, 685, 1995.
126. Waelti, E. R., Inaebit, S. P., and Rast, H. P., Co-culture of human keratinocytes on post-mitotic human dermal fibroblast feeder cells: production of large amount of interleukin-6, *J. Invest. Dermatol.*, 98, 805, 1992.
127. Kupper, T., Interleukin 1 and other human keratinocytes cytokines in inflammation and functional characterization, *Adv. Dermatol.*, 3, 293, 1988.
128. Cumberbatch, M. and Kimber, I., Dermal tumor necrosis factor alpha induces dendritic cell migration to draining lymph nodes and possibly provides one stimulus for Langerhans cell migration, *Immunology*, 75, 257, 1992.
129. Gueniche, A., Viac, J., Lizard, G., Chaveron, M., and Schmitt, D., Effect of different sensitizing haptens on intracellular adhesion molecule-1 expression and cytokine production by normal human keratinocytes, *Eur. J. Dermatol.*, 5, 320, 1995.
130. Bettley, F. R., The toxicity of soaps and detergents, *Br. J. Dermatol.*, 80, 635, 1968.
131. Singer, E. J. and Pitts, E. P., *Surfactants in Cosmetics*, Rieger, M., Ed., New York, 1985, 133.
132. Rowbottom, A. W., Norton, J., Riches, P. G., and Sloane, J. P., Cytokine gene expression in skin and lymphoid organs in human graft-versus-host disease, *J. Pathol.*, 169, 150A, 1993.
133. Anttila, H. S. I., Reitamo, S., Erkko, P., Ceska, M., Moser, B., and Baggolini, M., Interleukin-8 immunoreactivity in the skin of healthy subjects and patients with palmoplantar pustulosis and psoriasis, *J. Invest. Dermatol.*, 98, 96, 1992.
134. Corsini, E., Shubert, C., Marinovich, M., and Galli, C. L., Role of mitochondria in Tributyltin-induced interleukin-1-alpha production in murine keratinocytes, *J. Invest. Dermatol.*, 107, 720, 1996a.
135. Corsini, E., Bruccoleri, A., Marinovich, M., and Galli, C. L., Endogenous interleukin-1-alpha is associated with skin irritation induced by tributyltin, *Toxicol. Appl. Pharmacol.*, 138, 268, 1996b.
136. Arner, E. S. J., Bjornstedt, M., and Holmgren, A., 1-Chloro-2,4-dinitrobenzene is an irreversible inhibitor of human thioredoxin reductase — loss of thioredoxin disulfide reductase activity is accompanied by a large increase in NADPH oxidase activity, *J. Biol. Chem.*, 270, 3479, 1995.
137. Chabot-Fletcher, M., Breton, J., Lee, J., Young, P., and Griswold, D. E., Interleukin-8 production is regulated by protein kinase C in human keratinocytes, *J. Invest. Dermatol.*, 103, 509, 1994.