

Keratins and the Keratinocyte Activation Cycle

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In wound healing and many pathologic conditions, keratinocytes become activated: they turn into migratory, hyperproliferative cells that produce and secrete extracellular matrix components and signaling polypeptides. At the same time, their cytoskeleton is also altered by the production of specific keratin proteins. These changes are orchestrated by growth factors, chemokines, and cytokines produced by keratinocytes and other cutaneous cell types. The responding intracellular signaling pathways activate transcription factors that regulate expression of keratin genes. Analysis of these processes led us to propose the existence of a keratinocyte activation cycle, in which the cells first become activated by the

release of IL-1. Subsequently, they maintain the activated state by autocrine production of proinflammatory and proliferative signals. Keratins K6 and K16 are markers of the active state. Signals from the lymphocytes, in the form of Interferon- γ , induce the expression of K17 and make keratinocytes contractile. This enables the keratinocytes to shrink the provisional fibronectin-rich basement membrane. Signals from the fibroblasts, in the form of TGF- β , induce the expression of K5 and K14, revert the keratinocytes to the healthy basal phenotype, and thus complete the activation cycle. *J Invest Dermatol* 116:633–640, 2001

Epidermal keratinocytes have two alternative pathways open to them: differentiation and activation. In healthy epidermis, keratinocytes differentiate from the basal layer through squamous, granular, and cornified layers. This process has been described in several review articles recently (Eckert *et al*, 1997; Fuchs *et al*, 1997; Mischke, 1998; Tomic-Canic *et al*, 1998). From the perspective of this paper, we point out that the differentiation process can be affected by vitamins, such as retinoic acid and vitamin D3, and that the expressions of specific keratin genes have been often used as markers for basal *versus* differentiating cells: K5 and K14 are expressed in the basal layer, K1, K2, and K10 in the differentiating cells (reviewed in Schweizer, 1993). In response to epidermal injury, however, or in certain pathologic conditions such as psoriasis, an alternative pathway is open to keratinocytes, that of activation (reviewed in Barker *et al*, 1991; Nickoloff and Turka, 1993; Kupper and Groves, 1995; Tomic-Canic *et al*, 1998; Murphy *et al*, 2000). The activation process can be affected by growth factors and cytokines, such as interleukin-1 (IL-1), tumor necrosis factor α (TNF- α), transforming growth factor α (TGF- α), TGF- β , and interferon- γ (IFN- γ). The expression of specific keratin genes has been used as a marker for activated cells; characteristically,

activated keratinocytes express K6, K16, and K17 keratin proteins, distinct from the keratins of the healthy epidermis. Activated keratinocytes are hyperproliferative, migratory, change their cytoskeleton, augment the levels of cell surface receptors, and produce components of the basement membrane. These responses are essential for re-epithelialization of the injured area. Activated keratinocytes also produce paracrine signals to alert fibroblasts, endothelial cells, melanocytes, and lymphocytes, as well as autocrine signals targeted at neighboring keratinocytes. These responses are essential for orchestrating the actions of the surrounding cell types in repair of the injured tissue. The affected cell types, in turn, produce their own autocrine and paracrine signals, which modify the actions of activated keratinocytes. Eventually, having responded to the injury, keratinocytes receive a “de-activation” signal and revert to the normal differentiation pathway. The regulatory processes involved in keratinocyte activation and de-activation, as well as the concomitant changes in keratin gene expression, are coordinated by secreted growth factors and cytokines, produced both by the keratinocytes and by the surrounding cell types. These regulatory processes are the subject of this review.

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Abbreviations: ERK, extracellularly regulated kinase; IKK, I κ B kinase; IRAK, IL-1 receptor associated kinase; JAK, Janus activated kinase; MAPK, mitogen activated protein kinase; MEK, MAPK/ERK kinase; NIK, NF κ B inducing kinase; PKC, protein kinase-C; TAK, TRAF associated kinase; TRADD, TNF α receptor associated death domain; TRAF, TNF α receptor associated factor.

INITIATOR OF ACTIVATION: IL-1

In healthy epidermis, keratinocytes are not activated and they slowly proliferate in the basal layer and differentiate in the suprabasal layers. Being exposed to the surroundings, however, they must be prepared to respond very quickly to injury from the environment. Therefore, keratinocytes produce sentinel molecules ready to signal promptly that an injury has occurred and the tissue needs to become activated. Activated keratinocytes repair the tissue and eventually become deactivated, reverting to normal differentiation. This process, termed the keratinocyte activation cycle, is

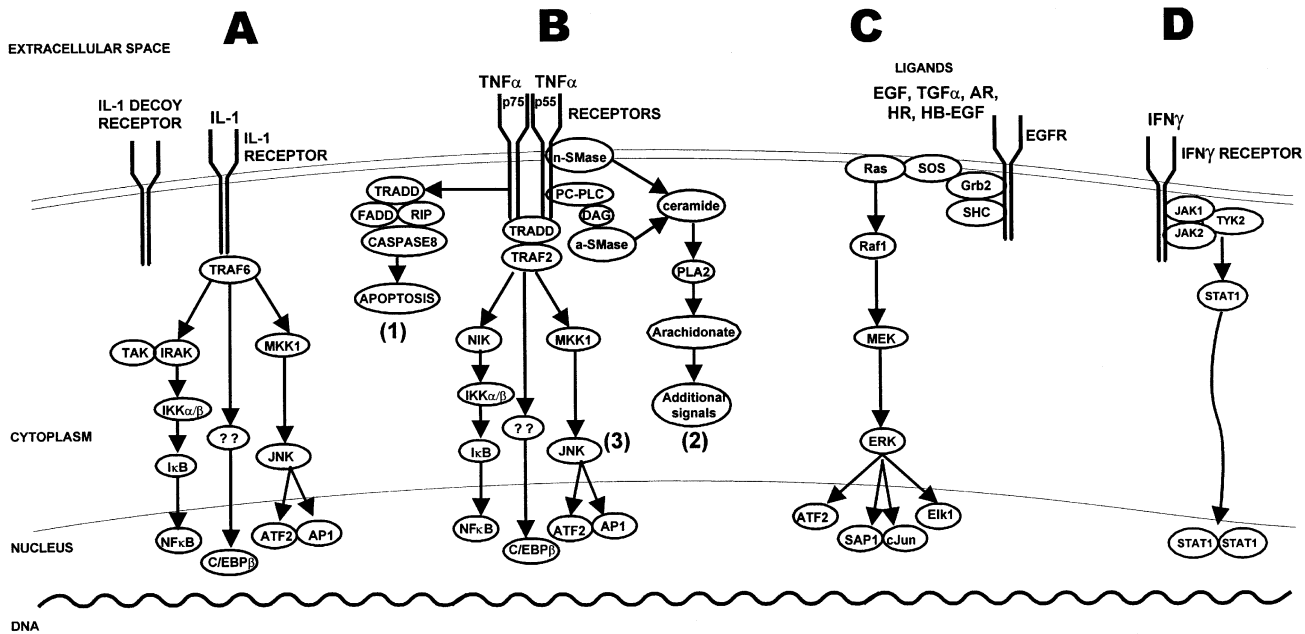


Figure 1. Signaling pathways in keratinocytes. (A) The IL-1 signal transduction pathways. The receptor interacts with TRAF6, which causes activation of protein kinases TAK, IRAK, and MKK1. This results in activation of transcription factors, such as NF κ B, C/EBP β , ATF2, and AP-1. (B) The TNF- α signal transduction pathways. There are three principal signal transduction pathways: (1) the apoptosis pathway; (2) the ceramide pathway; and (3) the TRAF2 pathway. The apoptosis pathway proceeds through a "death domain" containing proteins TRADD and FADD. In the ceramide pathway, PC-PLC stand for phosphatidyl-choline-activated phospholipase-C, DAG for diacyl-glycerol, n-SMase and a-SMase for neutral and acidic sphingomyelinase, and PLA2 for phospholipase-A2. TRAF2, via kinases NIK and IKKs, phosphorylates and causes subsequent degradation of I κ B, which allows NF κ B to become activated and enter the nucleus. TRAF2 also activates the MKK1 and JNK pathways. The mechanisms activating C/EBP β have not yet been elucidated. (C) The TGF- α signal transduction pathways. Growth factors, such as TGF- α , EGF, etc., bind to EGFR activating the cytoplasmic tyrosine kinase. Activated kinase binds scaffolding proteins, such as SHC, Grb2, and SOS, bringing them in the close proximity of Ras. They activate Ras, which activates Raf1, which activates MEKs, which activate ERKs. When activated, ERKs translocate to the nucleus, where they phosphorylate and thus activate transcription factors, such as ATF2, SAP1, c-Jun, and Elk1. (D) The IFN- γ signal transduction pathway. Binding of the ligand to the receptor causes its association with the JAK/TYK kinases, which phosphorylate STATs. STATs, when phosphorylated, dimerize and translocate to the nucleus where they activate transcription.

governed by extracellular signals, and is characterized by changes in expression of keratin proteins.

The most common initiator of keratinocyte activation is IL-1. Both the α and the β form of this cytokine are present unprocessed in the cytoplasm of keratinocytes. They are unavailable for binding to the cell surface receptors because they are sequestered in the cytoplasm (Hauser *et al*, 1986; Kupper *et al*, 1986a; Mizutani *et al*, 1991a; Kupper and Groves, 1995). Cytoplasmic IL-1 stands sentry in the epidermis, ready to respond to injury. Injured keratinocytes process and release IL-1, allowing the surrounding cells to perceive it (Kupper *et al*, 1986b; Murphy *et al*, 1989; Bochner *et al*, 1990; Mizutani *et al*, 1991b; Chan *et al*, 1992; Wood *et al*, 1996; Yu *et al*, 1996; Lundqvist and Egelrud, 1997; Zepeter *et al*, 1997; Corsini *et al*, 1998; Murphy *et al*, 2000). The released IL-1 serves as a paracrine signal to dermal endothelial cells to become activated, express selectins, and slow down the circulating lymphocytes (Cartwright *et al*, 1995; Lee *et al*, 1997; Romero *et al*, 1997; Wyble *et al*, 1997). IL-1 also serves as a chemoattractant for lymphocytes, causing them to extravasate and migrate to the site of injury (Nourshargh *et al*, 1995; Santamaria Babi *et al*, 1995). Furthermore, IL-1 is an activator of dermal fibroblasts, enhancing their migration, proliferation, and production of dermal extracellular matrix components (Mauviel *et al*, 1991; 1993; Godessart *et al*, 1994; Maas-Szabowski and Fusenig, 1996). IL-1 is also an autocrine signal that activates keratinocytes. IL-1 causes them to proliferate, become migratory, and express an activation-specific set of genes (Kupper, 1990a; Gyulai *et al*, 1994; Chen *et al*, 1995; Tomic-Canic *et al*, 1998).

Keratinocytes express IL-1 receptors, both the type I, functional, and the type II, decoy, on their surface, as well as the IL-1 receptor antagonist (Blanton *et al*, 1989; Stosic-Grujicic and Lukic, 1992; Kutsch *et al*, 1993; Eller *et al*, 1995; Grewe *et al*, 1996; Debets *et al*,

1997; Rauschmayr *et al*, 1997). The epidermal responses to IL-1 are exquisitely finely tuned: keratinocytes must be ready to respond quickly to injury via IL-1 and at the same time must be able to attenuate and shut off the IL-1 signals after the initial response.

Signal transduction in response to IL-1 starts at the cell surface with the type I receptor. The intracellular domain of this receptor associates with several proteins, e.g., TNF α receptor associated factor (TRAF)-6, which recruit protein kinases such as IL-1 receptor associated factor (IRAK) and TRAF associated kinase (TAK). Downstream from the kinases, the signal trifurcates and at least three transcription factor systems are activated: the NF κ B, C/EBP β , and AP-1 (Fig 1A) (Cao *et al*, 1996; Muzio *et al*, 1997; La and Greene, 1998; Baud *et al*, 1999; Lomaga *et al*, 1999; Ninomiya-Tsuji *et al*, 1999; Ling and Goeddel, 2000). These transcription factors then induce expression of the activation-specific proteins.

Among genes induced by IL-1 are growth factors and cytokines that transmit the signals of the specific type of injury to the surrounding cells. These include granulocyte-macrophage colony stimulating factor (GM-CSF), TNF- α , TGF- α , amphiregulin, additional IL-1, etc. (Kupper *et al*, 1988; Larsen *et al*, 1989; Tosato and Jones, 1990; Lyons *et al*, 1993; Lee *et al*, 1994; Chen *et al*, 1995; Lontz *et al*, 1995; Bechtel *et al*, 1996; Chung *et al*, 1996; Fujisawa *et al*, 1997a, b; Nylander-Lundqvist and Egelrud, 1997; Kozłowska *et al*, 1998). Activated keratinocytes also produce cell surface markers, such as intercellular adhesion molecule 1 (ICAM-1) and integrins as well as fibronectin, a component of the basement membrane that promotes keratinocyte migration (Kubo *et al*, 1984; O'Keefe *et al*, 1987; Griffiths *et al*, 1989; Lisby *et al*, 1989; Clark, 1990; Guo *et al*, 1991; Grinnell, 1992; Krutmann *et al*, 1992; Middleton and Norris, 1995).

Among the genes induced by IL-1 are keratins K6 and K16. Whereas the mechanism of induction of K16 is still under investigation, many details of the induction of K6 are known. Recently, we reported on the mechanism of induction of K6 by IL-1 (Komine *et al*, 2001). Skin biopsies in organ culture treated with IL-1 express K6 throughout the tissue. In cultures only confluent keratinocytes respond to IL-1; subconfluent cultures do not. Using DNA-mediated cell transfection, we identified the IL-1 responsive DNA element in the K6 promoter, and determined that it contains a complex of C/EBP binding sites. Thus, IL-1 initiates keratinocyte activation not only by triggering additional signaling events, but also by inducing directly the synthesis of K6 in epidermal keratinocytes, and thus changing the composition of their cytoskeleton.

MAINTENANCE OF ACTIVATION

Whereas IL-1 initiates the keratinocyte activation, other signals are used to maintain keratinocyte activation. Such signals need not be already present in healthy tissue and can have overlapping but different mechanisms of action from IL-1. Because these signals are not present in healthy tissue, keratinocytes do not need to elaborate sophisticated hair-trigger mechanisms to respond to or protect themselves from these signals. One such signal is TNF- α . Induced by IL-1, TNF- α can maintain keratinocytes in an activated state (Nickoloff and Turka, 1993).

TNF- α was discovered from two independent lines of research, first as an inducer of necrosis in some tumor cells and second as a cause of cachexia in septic animals. Subsequently, it was established that TNF- α is one of the proinflammatory cytokines that induce many inflammatory effects, such as fever and shock. In response to infection or injury a wide variety of cells produce TNF- α , primarily macrophages and monocytes but also epithelial cells including keratinocytes (Kock *et al*, 1990; Nickoloff *et al*, 1991; Kolde *et al*, 1992).

A low level of TNF- α is present in the upper layers of the healthy epidermis, but IL-1 can induce its synthesis and release from keratinocytes. The levels of TNF- α are greatly augmented under a variety of conditions, such as allergic and irritant contact dermatitis, infection, and ultraviolet irradiation (Barker *et al*, 1991). In these pathologic conditions TNF- α activates immune responses by inducing production of additional signaling molecules, cytokines, growth factors, their receptors, and adhesion proteins (e.g., amphiregulin, TGF- α , IL-1 α , IL-1 receptor antagonist, epidermal growth factor receptor (EGFR), and ICAM-1 (Griffiths *et al*, 1995, and references therein).

The signaling cascades mediating cellular responses to TNF- α have been partly elucidated (Rothe *et al*, 1994; 1995; Liu *et al*, 1996; Shu *et al*, 1996; Malinin *et al*, 1997; Natoli *et al*, 1997; Regnier *et al*, 1997; Song *et al*, 1997). The effects of TNF- α partly overlap those of IL-1, but the TNF- α -dependent signal transduction appears to be much more complicated than the IL-1-triggered one (although it is possible that at the moment we see too many trees, which perhaps obscures the forest). A current version of the cascade is shown in **Fig 1(B)**. There are two TNF- α receptors, but keratinocytes express mainly the 55 kDa receptor, type 1 (Trefzer *et al*, 1991; Kristensen *et al*, 1993; Kondo and Sauder, 1997). Three major intracellular effects are caused by TNF- α . The first is the induction of apoptosis, which proceeds through activation of caspases. The second involves production of ceramides, which in turn act as second messengers activating arachidonic acid synthesis and regulating downstream effects. Ceramides activate protein kinases that feed into the mitogen activated protein kinase (MAPK) cascade system. The third and most direct TNF- α signaling pathway involves proteins TNF α receptor associated death domain (TRADD) and TRAF2, which, through NF κ B inducing kinase (NIK) and other kinases, activate transcription factors NF κ B and C/EBP β . The same pathway activates members of the AP-1 transcription factor family. There is significant crosstalk between the TNF- α signaling and the MAPK cascade pathways.

The NF κ B family includes the proteins p65, p50, and c/rel, which both homodimerize and heterodimerize among themselves (Miyamoto and Verma, 1995). These proteins are stored latent in the cytoplasm, bound to the inhibitory protein I κ B. TNF- α causes activation of IKKs, kinases that phosphorylate I κ B and induce its degradation. The degradation of I κ B results in activation and nuclear translocation of the NF κ B protein (Beg *et al*, 1993; Shu *et al*, 1996; Regnier *et al*, 1997; Zandi *et al*, 1998). Knockout of IKK- α has a severe epidermal phenotype causing incomplete epidermal differentiation (Hu *et al*, 1999; Takeda *et al*, 1999). On the other hand, a knockout of IKK- β is defective in signaling from TNF- α to NF κ B (Li *et al*, 1999a; 1999b). NF κ B proteins can interact with C/EBP β , AP-1, and other transcription factors to regulate gene expression (Matsusaka *et al*, 1993; Stein *et al*, 1993). In keratinocytes, *in vitro* overexpression of NF κ B inhibits proliferation. In epidermis *in vivo* NF κ B is present in all layers, but is nuclear only in the suprabasal ones; this suggests a role for NF κ B in epidermal differentiation (Seitz *et al*, 1998). On the other hand, constitutive activation of NF κ B in I κ B-knockout mice results in normal epidermal development and differentiation, but a widespread and lethal dermatitis in the first few days of life (Klement *et al*, 1996).

TNF- α and other extracellular stimuli activate transcription factor C/EBP β (also known as NF-IL6 or LAP; Nakajima *et al*, 1993; Trautwein *et al*, 1993; Akira *et al*, 1997). The mechanisms that activate C/EBP β have not been fully characterized. C/EBP β interacts with many other transcription factors, such as the RB protein, the glucocorticoid receptor, Myc, NF κ B, and AP-1 (Brasier *et al*, 1990; Matsusaka *et al*, 1993; Nishio *et al*, 1993; Stein and Baldwin, 1993; Klampfer *et al*, 1994; Chen *et al*, 1996; Mink *et al*, 1996). In epidermis the C/EBP proteins are differentially expressed during differentiation (Maytin and Habener, 1998; Oh and Smart, 1998). Whereas knockout mice lacking C/EBP β have no cutaneous phenotype (Tanaka *et al*, 1995), overexpression of C/EBP β in keratinocytes causes growth arrest and induction of early differentiation markers (Zhu *et al*, 1999).

Using cultured keratinocytes and a novel *ex vivo* system, we showed that TNF- α induces the expression of K6 at the level of transcription (Komine *et al*, 2000). Using cotransfection, specific inhibitors, and antisense oligonucleotides, we have identified NF κ B and C/EBP β as the transcription factors that convey the TNF- α signal. Both are necessary for the induction and they apparently act as a complex, although only C/EBP β binds the K6 promoter DNA. The site in the K6 gene promoter that responds to TNF- α is separate from the site responsive to TGF- α . These results show that the inflammatory (TNF- α) and the proliferative (TGF- α) signals in epidermis regulate the expression of K6 separately and independently. Thus the cytoskeletal responses, such as K6 synthesis, can be precisely tuned in epidermal cells by separate proinflammatory and proliferative signals to fit the nature of the injuries that caused them.

Whereas IL-1 and TNF- α are proinflammatory signals with overlapping intracellular molecular pathways, under certain conditions keratinocytes need additional and different stimuli, which direct them to proliferate. In epidermis, several members of the EGF family can be produced, including TGF- α , amphiregulin, HB-EGF, and heregulin, ligands of the EGFR. These convey proliferative signals to keratinocytes.

Arguably the most extensively studied cellular receptor signaling pathways are those proceeding through EGFR (Ullrich and Schlessinger, 1990). In adult epidermis, EGFR is primarily expressed in the basal layer and, to a lesser degree, the first suprabasal layers (Nanney *et al*, 1990). Binding of the appropriate ligands to the EGFR can activate keratinocytes (Coffey *et al*, 1987). The signals activate nuclear proteins that regulate both gene expression and cell division. Among the regulated genes are those encoding additional regulators, leading to major morphologic changes, developmental changes, and differentiation. In response to the activation of the EGFR, keratinocytes proliferate, degrade

components of the extracellular matrix, and become migratory (Nickoloff *et al*, 1990).

A "simplified" scheme of the cascade is shown in **Fig 1(C)**. The binding of a ligand to EGFR causes the receptor to dimerize, with concomitant activation of its intracellular protein tyrosine kinase. A substrate for this kinase is the receptor itself – the two monomers phosphorylate each other. The phosphotyrosines serve as docking sites for SH2 domain containing proteins (such as Grb2 or SHC) that interact with proteins capable of activating Ras. Several growth factor receptors, *via* different adaptor molecules, activate Ras, which makes Ras a fulcrum for signal transduction pathways (**Fig 1C**). Activated Ras, in turn, activates a cascade of three protein kinases, Raf1, MAPK/ERK kinase (MEK), and extracellularly regulated kinase (ERK). The last one, ERK, translocates to the nucleus where it phosphorylates and thus activates transcription factors such as Elk1 and SAP1 (reviewed in Ullrich and Schlessinger, 1990; Hill and Treisman, 1995).

Successive activation of a cascade of three protein kinases, first characterized in the EGF/TGF- α signaling pathway, is a recurrent motif in signal transduction. Stress, exemplified by osmotic shock and ultraviolet irradiation, or proinflammatory cytokines including TNF- α and IL-1, can activate parallel cascades (see above), thus activating partially overlapping sets of transcription factors (Dérjard *et al*, 1994; Galcheva-Gargova *et al*, 1994; Gupta *et al*, 1995; 1996; Rosette and Karin, 1995). All these cascades are present and functional in keratinocytes (M.B. unpublished).

Perhaps the best-characterized TGF- α -responsive transcription factors are those belonging to the AP-1 family. AP-1 is a nuclear transcription complex composed of dimers encoded by the *fos* and *jun* families of proto-oncogenes (Hill and Treisman, 1995; Karin, 1996). Whereas Fos proteins only heterodimerize with members of the Jun family, Jun proteins can dimerize with both Fos and other Jun proteins. In the epidermis, AP-1 regulates cell growth, differentiation, and transformation (Bernerd *et al*, 1993; Saez *et al*, 1995; Rutberg *et al*, 1996). The expression of individual AP-1 proteins in epidermal layers, however, is a controversial issue that awaits resolution. Certain authors find c-Fos in lower layers of the epidermis (Fisher *et al*, 1991; Basset-Seguín *et al*, 1994; Lu *et al*, 1994) whereas others do not find any c-Fos (Rutberg *et al*, 1996), which agrees with the lack of an epidermal phenotype in *c-fos* knockout mice (Saez *et al*, 1995). The differing results could be explained by different epitopes of the antibodies used and functional redundancy of Fos family members. Be that as it may, it is clear that the AP-1 proteins in keratinocytes can regulate the expression of differentiation markers (Presland *et al*, 1992; Lu *et al*, 1994; Lohman *et al*, 1997) and may convey the calcium- and protein kinase C (PKC) dependent signals (Welter *et al*, 1995; Rutberg *et al*, 1996). Functional AP-1 sites have been found in many keratin genes, including the first intron of human and murine K18 and the K8 gene (Pankov *et al*, 1994; Umezawa *et al*, 1997). We have found that the EGFR ligands strongly and specifically induce the expression of K6 and K16 and that AP-1 sites are present and functional in several epidermal keratin genes (Jiang *et al*, 1993; Ma *et al*, 1997).

THE ACTIVATED PHENOTYPE

Once activated, keratinocytes synthesize additional signaling growth factors and cytokines including TGF- α , IL-3, IL-6, IL-8, G-CSF, GM-CSF, and M-CSF (Coffey *et al*, 1987; Kupper, 1990b; Nickoloff *et al*, 1990). The effects of these signaling molecules produced by keratinocytes are chemotactic for white blood cells and paracrine for lymphocytes, fibroblasts, and endothelial cells. Interestingly, these signaling molecules are also autocrine for keratinocytes themselves. They lead to secondary effects of keratinocyte activation. Several extracellular markers are specifically expressed by the activated keratinocytes. These include cell surface proteins, integrins, components of the extracellular matrix, as well as receptors for both the autocrine factors and factors produced by the infiltrating immune cells (Alitalo *et al*, 1982; O'Keefe *et al*,

1987; Marinkovich *et al*, 1992; Burgeson, 1993). In a feedback loop, the increase in the expression of cell surface receptors may augment the initial activation signal. The various signaling molecules may be synergistic or antagonistic with each other. This allows the activated phenotype to be specifically modified, which can lead to different activated phenotypes. Put simply, keratinocytes activated during wound healing, in psoriasis, or other pathologic conditions can have different variants of the activated keratinocyte phenotype.

THE CONTRACTILE KERATINOCYTE: IFN- γ

In the late stages of wound healing, the contraction of the newly formed extracellular matrix produced by the fibroblasts is an important process. This contraction is effected by fibroblasts; however, keratinocytes have their own task, to contract the newly deposited, fibronectin-rich basement membrane. The signal that compels keratinocytes to become competent to contract is, apparently, IFN- γ .

The most extensively studied signaling molecules of the immune system are the interferons IFN- α , IFN- β , and IFN- γ , a subset of cytokines originally described as factors that protect cells from viral infections (reviewed in Schindler and Darnell, 1995). IFN- α and IFN- β share a cell surface receptor, whereas IFN- γ binds to a different receptor and has distinct effects. Certain diseases, such as psoriasis, are associated with high levels of IFN- γ in epidermis (Nickoloff *et al*, 1990). Although the role of interferons in pathologic processes has not been clearly defined, they have been used in therapeutic trials for several dermatologic diseases (Eron *et al*, 1987).

Activation of IFN receptors initiates a cascade of protein phosphorylation events. The cascade branches into a delta transcription activating pathways that induce multiple genes (Schindler and Darnell, 1995). The receptors interact with Janus activated kinases (JAK) kinases, which phosphorylate tyrosines both on the receptors and on the signal transducing activator of transcription (STAT) family of transcription factors (**Fig 1D**). First discovered as mediators of interferon signaling, STATs are unusual because they can convey the signal directly from the plasma membrane into the nucleus without second messengers or cytoplasmic kinase cascade intermediates (Levy and Darnell, 1990). Each STAT contains a tyrosine phosphorylation site and an SH2 domain that can bind to phosphotyrosine. STATs are cytoplasmic in their ground state, but upon activation of appropriate receptors they become phosphorylated and, through their SH2 domains, dimerize and translocate into the nucleus. In the nucleus STATs bind to specific DNA recognition elements and activate transcription of nearby genes. To date six STAT proteins have been characterized; they are activated by a variety of extracellular stimuli. The regulatory specificity of the cytokine signals at the cell surface is mirrored in the nucleus by the activity of specific members of the STAT family: IFN- γ leads to activation of STAT-1, IFN- α of STAT-2 and STAT-3, IL-6 and OSM of STAT-3, IL-12 of STAT-3 and STAT-4, IL-3, IL-5, and GM-CSF of STAT-5, and IL-4 of STAT-6 (Schindler and Darnell, 1995).

We found that IFN- γ strongly and specifically induced the promoter of the K17 gene. No other keratin gene construct was induced (Jiang *et al*, 1994). Within the promoter of the K17 gene, we have identified and characterized a site that confers the responsiveness to IFN- γ , and that binds the transcription factor STAT-1. We could induce *in vivo* expression of K17 experimentally by causing a delayed-type hypersensitivity inflammatory reaction characterized by substantial infiltration of lymphocytes that produce IFN- γ (Kaplan *et al*, 1986). In affected epidermis, we found transcription factor STAT-1 in the nuclei of keratinocytes. In contrast, STAT-1 is cytoplasmic in unaffected and healthy skin.

Psoriasis is a Th-1-dependent process that is associated with production of IFN- γ . We hypothesized that the induction of K17 is specific for Th-1 inflammatory reactions and does not occur in Th-2 type ones. Therefore, we analyzed lesional samples of psoriasis

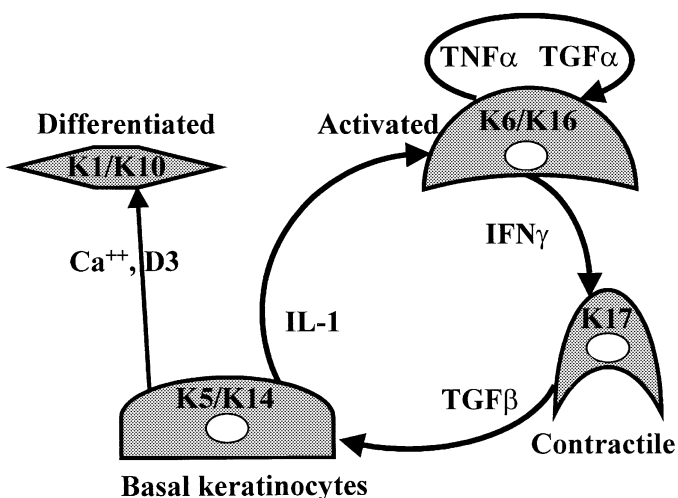


Figure 2. The keratinocyte activation cycle. Basal keratinocytes, producing K5 and K14, can either differentiate and produce K1 and K10, or become activated, producing K6 and K16. IL-1 is the primary signal initiating keratinocyte activation and expression of K6 and K16. TNF- α and TGF- α keep keratinocytes activated until another signal, such as IFN- γ , is received. IFN- γ induces K17 and promotes contractility in keratinocytes. TGF- β is a de-activating signal that promotes reversal to the basal phenotype and induces expression of K5 and K14.

and compared them with those of atopic dermatitis, a Th-2-associated process. The above hypothesis has been supported by our evidence that K17 is induced in the first, but not in the second, disorder (Komine *et al*, 1996). Our data further indicated that Th-1 and Th-2 lymphocytes, through the cytokines they produce, differently regulate not only each other, but also keratin gene expression in epidermis, their target tissue (Komine *et al*, 1996). These results characterize, at the molecular level, a signaling pathway produced by the infiltration of lymphocytes in skin and resulting in the specific alteration of gene expression in keratinocytes. They define at the molecular level how IFN- γ regulates expression of the K17 gene and provide a means for analysis of the molecular interactions between the immune system and the epidermis, interactions that are important in pathologic skin processes (Komine *et al*, 1996).

K17 is exceptional because it is not found in healthy interfollicular epidermis, but it is expressed in certain pathologic states, including psoriasis, allergic reactions, and cutaneous T cell lymphoma, as well as in benign tumors of the mammary gland, basal cell epitheliomas, squamous cell lung carcinomas, and some other benign and malignant neoplasms (Moll *et al*, 1984; Guelstein *et al*, 1988; de Jong *et al*, 1991; Wetzels *et al*, 1991; Blumenberg, 1994; Jiang *et al*, 1994). Indeed, expression of K17 has been used to evaluate the course of treatment of psoriatic patients (de Jong *et al*, 1991).

K17 is expressed in various healthy epithelia (Trojanovsky *et al*, 1992), including myoepithelial cells, basal layers of transitional and pseudostratified epithelia of the respiratory and urinary tracts, and early developmental stages of stratified epithelia. Common characteristics of these cells are contractility and/or frequent changes in shape (Trojanovsky *et al*, 1992). The function of K17 in epidermis therefore may be to promote or allow keratinocyte contractility.

BACK TO BASICS: TGF- β

Once the injury that causes keratinocyte activation has been healed and the tissue repaired, keratinocytes must revert to their regular function, differentiation into stratum corneum. To revert to the basal cell phenotype, keratinocytes need a signal that the injury is over. This signal comes from the dermal fibroblasts in the form of TGF- β .

TGF- β is an important regulator of epidermal keratinocyte function because it suppresses cell proliferation, whereas it induces

synthesis of extracellular matrix proteins and their cell surface receptors. Mice with a knocked-out TGF- β gene develop normally, because of the maternally supplied TGF- β , only to succumb to exuberant multifocal inflammation due to unrestrained activation of the immune system (Shull *et al*, 1992; Geiser *et al*, 1993). Skin-targeted overexpression of TGF- β causes hypoplasia, whereas loss of TGF- β expression or resistance to TGF- β cause increased susceptibility to malignant conversion (Jhappan *et al*, 1993; Glick *et al*, 1993; Pierce *et al*, 1993; Reiss *et al*, 1993; Sellheyer *et al*, 1993).

In skin, TGF- β induces expression of extracellular matrix and basement membrane components, such as fibronectin, laminin, and collagen IV and VII (Wikner *et al*, 1988; Ryyänen *et al*, 1991; Vollberg *et al*, 1991; König and Bruckner-Tuderman, 1992), extracellular proteases and their inhibitors (Edwards *et al*, 1987; Laiho *et al*, 1987; Salo *et al*, 1991; Keski-Oja and Koli, 1992), as well as cell surface proteins including integrins α 5, α v, β 1, β 4, and β 5, and bullous pemphigoid antigens BPAG1 and BPAG2 (Vollberg *et al*, 1991; Gailit *et al*, 1994). We have shown that TGF- β specifically induces synthesis of basal-cell-specific K5 and K14 (Jiang *et al*, 1995).

Overall, it appears that TGF- β promotes the synthesis of basal-cell-specific proteins and therefore promotes the basal phenotype. This happens at the expense both of the activated, hyperproliferative phenotype and of the differentiating phenotype. Our conclusion is strengthened by studies showing that the keratinocyte growth arrest by TGF- β is reversible, does not result in terminal differentiation, and can be modulated by regulators of keratinocyte differentiation, such as retinoic acid or calcium (Choi and Fuchs, 1990; Matsumoto *et al*, 1990; Wang *et al*, 1992). Furthermore, van Ruissen *et al* (1994) have shown, by using careful cytometric measurements, that *in vitro* TGF- β reduces the fast growth rate of keratinocytes to the slow level of cell division observed in the normal, nonhyperproliferative basal layer of skin *in vivo*. From these data we suggest that the effects of TGF- β on keratinocytes are not antiproliferative, but antihyperproliferative.

OVERVIEW

When we put all these data together, we arrive at a consistent framework for the action of growth factors and cytokines in epidermal injury (Fig 2). The first signal from the injury is the release of IL-1. This release activates endothelial cells and fibroblasts and invites lymphocytes to the wound site. At the same time, IL-1 activates keratinocytes, making them hyperproliferative and migratory, causing them to deposit a provisional fibronectin-rich basement membrane, express K6 and K16, and produce additional growth factors and cytokines, including TNF- α and members of the EGF family. These growth factors and cytokines maintain the keratinocytes in the activated state. Meanwhile, lymphocytes extravasate and migrate to the wound site to fight any infection and produce IFN- γ . IFN- γ is an autocrine signal activating the lymphocytes, but it is also a paracrine signal to keratinocytes, communicating the following message: "the infection is being dealt with; if the re-epithelialization is complete, it is time to express K17, to contract and reorganize the provisional basement membrane". Meanwhile, fibroblasts migrate to the wound site, producing extracellular matrix, expressing TGF- β . TGF- β is an autocrine signal activating the fibroblasts, but it is also a paracrine signal to keratinocytes, communicating the following message: "the dermis is being repaired; it is now time to start producing K5 and K14, to return to being a basal cell and the process of normal differentiation".

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