The activated keratinocyte

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Abbreviations: **bFGF**: basic fibroblast growth factor; **ET-1** endothélin-1; **GM-CSF**: granulocyte macrophage colony stimulating factor; **Gro**: growth-related oncogene; **ICAM**: intercellular cell adhesion molecule; **IFN**: interferon; **IL**: interleukin; **IP**: interferon-γ inducible protein; **MCP**: monocyte chemotactic protein; **Mig**: monokine induced by interferon-γ **MIP**: macrophage inflammatory protein; **NGF**: neuronal growth factor; **PDGF**: platelet derived growth factor; **RANTES**: regulated on activation normal T cell expressed and secreted; **SCF**: stem cell factor; **TARC**: thymus and activation-regulated chemokine, **TGF**: transforming growth factor; **TNF**: tumor necrosis factor; **TSLP**: thymic stromal lymphopoietin; **VEGF**: vascular endothelial growth factor

Keratinocytes, the main constituent cells of the epidermis are key cells in the initiation and maintenance of the inflammatory state in the skin. The increased inflammatory function of the keratinocytes is acquired after exposure to a variety of physical and chemical stimuli present in the microenvironment. Keratinocytes are particularly reactive to acute perturbation of cutaneous permeability barrier. The nature of the stimuli can be quite diverse ranging from contact allergens and UV light (external stimuli) to cytokines derived from inflammatory cells (internal stimuli). Potent stimuli of keratinocytes are for instance interferon-γ, derived from activated T cells and TNFα produced by monocytes. The activation process can also be affected by growth factors such as TGFα, TGFβ which can stimulate the expression of specific keratin genes. In wound healing, activated keratinocytes also express keratin proteins distinct from the keratins of the healthy epidermis and are hyperproliferative, migratory; they augment the levels of cell surface receptors and produce components of the basement membrane. Activated keratinocytes produce paracrine signals to alert fibroblasts, endothelial cells, melanocytes, lymphocytes as well as autocrine signals targeted at neighboring keratinocytes. All these responses orchestrate the actions of the surrounding cell types in repair of the injured tissue. The regulatory processes involved in keratinocyte activation are coordinated by secreted growth factors and cytokines produced both by the keratinocytes and the surrounding cells.

Activated keratinocytes release cytokines

In healthy epidermis, keratinocytes are not activated. Resting or unstimulated keratinocytes express and secrete low levels of cytokines but have a reservoir of preformed cytokines such as IL-1 which is the most common initiator of keratinocyte activation.
The inflammatory function of keratinocytes becomes manifest by the production of a broad range of cytokines which interplay with other locally produced inflammatory mediators to result in the recruitment of leucocytes creating a skin infiltrate. The release of IL-1 activates endothelial cells and fibroblasts and produces additional growth factors and cytokines which maintain the keratinocytes in the activated state.
Moreover, a superfamily of cytokines with leucocyte chemotactic properties and called chemokines have been reported in the epidermis after appropriate stimulation. Chemokines have been divided into two major subfamilies on the basis of the arrangement of the two N-terminal cysteine residues, CXC and CC, depending on whether the cysteine residues have an amino acid between them (CXC) or are adjacent (CC).
Activated keratinocytes can thus release a wide panel of cytokines including ***interleukins*** (IL-1, IL-6, IL-7, IL-7-like or TSLP, IL-8, IL-10, IL-12, IL-15, IL-18, IL-20...), ***growth factors*** (GM-CSF, NGF, TGFα,β, PDGF, bFGF, SCF, amphiregulin, endothelin-1, VEGF...)...), ***chemokines*** (IL-8/CXCL8, GRO-α /CXCL-1, IP-10/CXCL10, MIG/CXCL-9, I-TAC/CXCL11, MCP-1/CCL2, RANTES/CCL5 MIP-3α/CCL20, CTACK/CCL27....), ***interferons*** (IFN-α,β), and ***tumor necrosis factor*** (TNF-α,β) which exert different effects on both epidermal and dermal skin cells which express specific receptors. These mediators influence the major cell types within the skin via this system of intercellular communication. It is noteworthy that the expression of most epidermal cytokines and chemokines varies with the stage of keratinocyte differentiation. Among cytokines, the production of IL-1, -6, -8 and TNFα is the best known. Keratinocyte-derived IL-7 and IL-15 likely play a role in T-cell trafficking and in the pathogenesis of cutaneous T-cell lymphoma. Keratinocytes were recently recognized as beeing source of other IL-10 family members like IL-20 but their role remains to be investigated.

The signaling cascade mediating cellular responses to cytokines have been partly elucidated mainly for IL-1 and TNFα. Signal transduction in response to cytokines starts at the cell surface with specific receptors. The intracellular domain of these receptors associates with proteins which recruit protein kinases and further activates transcription factor systems (NFκB, AP-1, AP-2....). These transcription factors then induce expression of the activation-specific proteins. Activated keratinocytes also produce cell surface markers such as intercellular adhesion molecule ICAM-1/CD54, integrins, fibronectin....ICAM-1, a ligand of the leucocyte function-associated antigen LFA-1 facilitates the binding and the recruitment of T lymphocytes into the epidermis.

Epidermal cell-derived cytokines and chemokines play an important role for the spatial distribution of immune cells

Epidermal cell-derived cytokines and chemokines initiate and regulate Langerhans cell migration

Langerhans cells (LC) are specialized antigen-presenting cells that reside in the epidermis as sentinels of the immune system. Their mobilization and their directed migration from the epidermis to draining lymph nodes are processes of pivotal importance in the generation of cutaneous immune responses. These processes are orchestrated by interactions between cytokines and chemokines and their respective receptors. Tissue injury and other perturbants of epidermal homeostasis provide danger signals leading to a local production of proinflammattory cytokines that induce LC mobilization to the lymphoid tissue. At the same time, signals are generated that recruit LC precursors into the skin to maintain the epidermal LC population.

Following skin sensitization, a proportion of epidermal LC are stimulated to leave the skin and to migrate, via afferent lymphatics to draining lymph nodes where they accumulate as immunostimulatory dendritic cells. Pro-inflammatory cytokines including IL-1β and TNFα are important signals for the initiation of this response. They promote LC emigration from the epidermis whereas the anti-inflammatory cytokine IL-10 is a counter-regulator.

IL-10 also inhibits the functional activity of LC and dendritic cells, in part due to regulation of the expression of costimulatory molecules. Recently, it has been demonstrated that IL-18, like IL-1β, contributes to the regulation of LC migration and a marked increase in lymph node dendritic cell numbers. GM-CSF and TGFβ1 also induce the migration of LC.

Vascular endothelial growth factor (VEGF) which is known to induce endothelial cell proliferation, promote cell migration, stimulate chemotaxis of monocytes and is also a chemokinetic factor for LC. Its production by keratinocytes is increased after contact with haptens, UVB exposure and in various inflammatory skin lesions. Its up-regulation by IFNγ in keratinocytes suggests an important role of this growth factor in the deregulated angiogenesis associated with inflammatory skin reactions characterized by a T-helper type 1 cell-mediated response (psoriasis).

Among chemokines, MIP-3α/CCL20 is a strong chemoattractant for CD1a+ LC precursors and LC express the sole MIP-3α receptor CCR6. This suggests that MIP-3α is involved in the selective LC recruitment into the epidermis.

Epidermal cell-derived cytokines and chemokines regulate the recruitment of leukocytes into inflamed skin

The migration of mononuclear cells to sites of skin inflammation is initiated by the ability of cytokines (IL1, TNFα...) to up-regulate the adhesion molecules on the dermal vascular endothelial cells. The recruited T-cells can in turn release IFNγ and other cytokines (IL-4,

TNFα ...) that can activate mast cells , macrophages, keratinocytes. Epidermal cells have been shown to be more sensitive to Th1 than to Th2-derived lymphokines in terms of chemokine release.
Induced and inflammatory chemokines IP-10/CXCL10 and Mig/CXCL11 mainly expressed by basal keratinocytes play a significant role in the recruitment and maintenance of activated T-cells which express CXCR3, in diverse skin diseases (psoriasis, lichen planus...).
IP-10 and IL-8 are consistently upregulated in the epidermis of patients with psoriasis but not in lesions of patients with atopic dermatitis suggesting that keratinocytes from these patients show an intrinsically abnormal and different chemokine production profile and may thus favor the recruitment of distinct leukocyte subsets into the skin.
TARC/CCL17, a ligand for CCR4 mainly expressed on Th2-type cells, is expressed by keratinocytes activated by TNFα and IFNγ which act synergistically but is inhibited by IL-4.
Recently, an IL-7-like cytokine called thymic stromal lymphopoietin (TLSP) has been involved in the induction of allergic inflammation. TSLP potently activates human CD11c+ dendritic cells which subsequently primed naïve Th cells to produce high concentrations of proallergic cytokines including IL-4, IL-5, IL-13, and TNFα, while downregulating IL-10 and IFNγ. TSLP is highly expressed by epithelial cells, especially keratinocytes of atopic dermatitis. TSLP espression is associated with LC migration and activation and thus represents a keratinocyte-derived cytokine that directly triggers dendritic cell-mediated allergic inflammation.

Activated keratinocytes exhibit an antimicrobial activity

Skin is constantly exposed to environmental microorganisms and exhibits an innate ability to fight invading microbes. The innate immune system of human skin contains antimicrobial peptides known as cathelicidins (LL37) and beta-defensins (hBD1, hBD2, hBD3). In normal skin, these peptides are negligible but they accumulate in skin affected by inflammatory diseases such as psoriasis. They are expressed by differentiated keratinocytes activated by inflammatory stimuli (IL-1, TNFα, IFNγ...). Antimicrobial peptides can alert the adaptive immune system. LL37 attracts neutrophils, monocytes and certain T cells. The constitutively expressed hBD-1 and the inducible hBD-2 and hBD-3 are also chemoattractants: hBD-2 selectively attracts the memory subset of peripheral T cells (CD4+/CD45RO+) and immature dendritic cells through CCR6 which also recognize MIP-3α.
Immunohistochemical analysis have confirmed the presence of abundant LL37 and hBD-2 in the superficial epidermis of psoriasis. In comparison, these peptides are decreased in acute and chronic lesions of atopic dermatitis and this deficiency may account for the susceptibility of patients with atopic dermatitis to skin infections.

Anti-inflammatory drugs and keratinocyte activation markers

Glucocorticoids represent one of the most widely used drugs in dermatology for their anti-inflammatory and immunosuppressive effects. They are described to inhibit the expression of multiple inflammatory genes likely through a direct inhibitory interaction between activated glucocorticoid receptors and activated transcription factors such as NFκB and AP-1 which regulate the inflammatory gene expression. They may also increase the transcription of genes coding for anti-inflammatory proteins (lipocortin-1, IL-1ra....). In UV-activated keratinocytes they reduce the expression of IL-6 and IL-8 but induce an increase of IL1-α in the presence of haptens or irritants.
Many drugs have been reported to modulate keratinocyte activation. Cetirizine and hydrocortisone differentially regulate ICAM-1 expression and chemokine release in cultured

human keratinocytes. Vitamin D3 analogues suppress IL-8 and IL-6 production by activated keratinocytes.
Zinc can reduce keratinocyte activation state such as ICAM-1 induction and TNFα production. Some monosaccharides have been also reported to exhibit inhibitory effects on lymphokine activity and exert a suppresive effect on the activation state of epidermal cells. More recently, flavonoids have been reported to inhibit IFN-γ-induced ICAM-1. Conversely, imiquimod and its analogs are cytokine inducers and enhance the cutaneous immune response.
It is noteworthy that neuropeptides may exert either proinflammatory or anti-inflammatory effects. Many of these neuropeptides are not only released by nerve cells but also by different cell types including keratinocytes. Proopiomelanocortin gene products such as adrenocorticotropin (ACTH) and α-melanocyte-stimulating hormone (α-MSH) which are produced by epidermal cells are potent immunomodulators that regulate the function and production of many cytokines. Activated keratinocytes release α-MSH which inhibits the production of IL-1 and is a potent inducer of IL-10.

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