Review Article

The role of keratinocytes in inflammation

Jana Juráňováa,b, Jana Frankováa,b,* and Jitka Ulrichováa,b

*Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic.

aInstitute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic.

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ABSTRACT

The epidermis is the external layer of the skin and is composed mainly of keratinocytes. Therefore, keratinocytes play an indispensable role as inherent constituents of the skin barrier in physical defenses against environmental threats. Keratinocytes also exert an active protective role against invasion by pathogens. This competency is of particular importance when physical defenses fail as a consequence of skin injury. During the inflammatory phase of healing, keratinocytes act as immuno-modulators, managing inflammation via a rigorously coordinated network of inflammatory cascades, triggered by keratinocyte-receptor communication with the surroundings in a paracrine and autocrine manner. This review summarizes current understandings of the coordinated inflammatory network and focuses on recent progress regarding the role of keratinocytes in early phases of skin wound healing.

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Introduction

A key objective of skin, as the largest peripheral organ, is to create a protective barrier against the external environment. Injury to skin tissue constitutes a crucial deterioration of this protective ability and leaves the body vulnerable to infection. Therefore, concerns about skin wound healing to restore skin integrity for human health have been expressed since ancient times (Reinke et al., 2012). In the 5th century BC, Hippocrates already emphasized the pivotal role of inflammation, when he pointed out the importance of draining pus from the wound ("Ubi pus, ibi evacua") (Eming et al., 2007). It is generally accepted that the role of inflammation lies in elimination of infection caused by invading pathogens and necrotic tissue in the wound bed. However, scarless healing of wounds to fetal skin that lacks the common inflammatory response, provides clear evidence that inflammation is an underlying prerequisite for scarring. Therefore, the question of whether inflammation is mandatory for successful healing remains a subject of controversy (Redd et al., 2004).

The epidermis is the upper layer of the skin, consisting almost entirely of keratinocytes. Thus, keratinocytes are in direct contact with the environment and form the first line of defense against environmental threats. For many years, the one and only assignment of keratinocytes was considered to be a physical barrier of the skin. Currently however, keratinocytes are regarded as active cells contributing to preservation of the immune barrier (Suter et al., 2009). In the intact epidermis, resting keratinocytes exert basal anti-inflammatory actions by the production of...
antimicrobial peptides (Soong et al., 2015). As a result of injury-induced imbalance, keratinocytes release pre-stored and newly formed acute phase proteins that challenge other skin cell types, as well as keratinocytes. Newly arriving neutrophils destroy bacteria and eliminate inflammation in the wound area, thus enabling successful healing (Soong et al., 2015; Weinheimer-Haus et al., 2015).

This review summarizes current views on keratinocytes as sensors of infection and powerful producers of key inflammatory mediators that alert skin cells to danger. It also provides new insights into interconnected pathways that regulate the inflammatory response, helping to identify potential targets for anti-inflammatory therapies.

**Acute inflammation in response to injury**

Acute skin inflammation may arise in response to physical wounding, UV irradiation, chemical irritants or exposure to allergens. Normally, this biological response is resolved within two weeks with no adverse effects on the tissue. Inflammation is a first stage of the wound healing process and is artificially divided into three overlapping phases: inflammatory phase (hemostasis and inflammation), proliferative phase (tissue formation) and maturation (tissue remodeling). Successful skin repair requires healing to be precisely timed and molecularly regulated (Eming et al., 2007; Singer and Clark, 1999).

In direct response to injury, platelets and extravasated leukocytes captured in a nascent hemostatic plug release growth factors and cytokines such as interleukins IL-6, IL-1α, IL-1β, interferon gamma (IFN-γ) and tumor necrosis factor-α (TNF-α) to initiate the inflammatory process (Reinke and Sorg, 2012).

**Activation and active role of keratinocytes during inflammation**

Exposure to pro-inflammatory cytokines, as well as a wound-generated electric field and loss of contact inhibition, results in activation of keratinocytes (Behm et al., 2012; Freedberg et al., 2001; Koivisto et al., 2011). Activated keratinocytes switch from their inactive status to a migratory, proliferative and pro-inflammatory phenotype. This shift is associated with alterations in cytoskeletal proteins (keratins) and expression of transmembrane receptors (integrins), as well as production and deposition of extracellular matrix components, including laminin-332 (Komine et al., 2000). Thus, the keratinocytes in the immediate neighborhood of the wound area lose their adhesive properties and begin to migrate over a provisional matrix; the keratinocytes behind the actively migrating cells start to proliferate (Hopkinson et al., 2014; Santoro and Gaudino, 2005).

Injured keratinocytes release the first signals, known as alarmins, consisting of high-mobility group box protein 1 (HMGB1), heat shock protein (HSP), antimicrobial peptides (defensins, cathelicidin, calgranulin A/B), cytokines (IL-1α, IL-33) and chemokines (IL-8) (Eckhart et al., 2013). These endogenous molecules are considered to be a subgroup of host-derived damage-associated molecular patterns (DAMPs) that signal tissue and cell injury via Toll-like receptors (TLRs), which, in turn, initiate immune responses (Bianchi, 2007; Lessard et al., 2013; Oppenheim and Yang, 2005). Activation of the TLR signaling pathway leads particularly to nuclear factor kappa-light chain enhancement of activated B cell (NF-κB) nuclear translocation and transcription of downstream target genes such as pro-inflammatory cytokines IL-1 family, IL-6, TNF-α, chemokine IL-8 or the enzyme cyclooxygenase-2 (COX-2) (Freedberg et al., 2001; Suter et al., 2009). The secretion of these pro-inflammatory molecules attracts neutrophils into the wound area, enabling the elimination of infectious agents. This is necessary to reestablish the epidermal barrier during wound healing (Takazawa et al., 2015). In addition, TNF-α and IL-1 can provide positive feedback to NF-κB and amplify the inflammatory response (Feldmeyer et al., 2010).

Following injury, the intracellular sensing of infection or stress by the nucleotide-binding oligomerization domain (Nod)-like receptor (NLR) promotes assembly and activation of an inflammasome. This multiprotein complex is involved in maturation of pro-inflammatory cytokines and thus mediates inflammation (Lee et al., 2015a). In the early stages of wound healing, keratinocytes express components of the NLR pyrin domain containing 3 (NLRP3) inflammasomes (Feldmeyer et al., 2010). This leads to activation of the protease procaspase-1, the key constituent of the inflamma-some that contributes to inflammation via proteolytic processing of IL-1β or IL-18 (Weinheimer-Haus et al., 2015) and most likely IL-33 (Keller et al., 2008; Ogura et al., 2006) or IL-1–α (Gross et al., 2012; Keller et al., 2008; Lee et al., 2015a). Subsequently, pleiotropic inflammatory cytokines of the IL-1 family mediate NF-κB signal transduction by binding to its receptor (Rauschmayr et al., 1997).

In summary, keratinocytes are capable of rearranging their own receptors and thus can adapt to diverse stress conditions by modulating their pro-inflammatory actions. Pro-inflammatory mediators that originate from activated keratinocytes may also act as paracrine regulators of fibroblasts, endothelial cells and immune cells, or in an autocrine fashion, potentiating the inflammatory response in keratinocytes themselves. These intertwined feedback loops of inflammatory events exemplify the complex role of keratinocytes in regulating inflammation (Suter et al., 2009).

**Modulation of keratin expression in activated keratinocytes**

Keratins represent a large family of cytoskeletal proteins that form intermediate filaments in epithelial cells (Komine et al., 2001). Under non-pathological conditions, basal keratinocytes attached to the basement membrane by hemidesmosomes slowly proliferate and express intermediate filament proteins: keratin 5, K14 and K15 (Freedberg et al., 2001). Detachment of keratinocytes from the basement membrane causes a shift to an alternative transcriptional profile through transcription factor p63 activity (Eckhart et al., 2013). In this sense, mature cells in the suprabasal layers express keratins K1 and K10, which are characteristic markers of differentiation (Freedberg et al., 2001; Luo et al., 2011; Usui et al., 2008).

Upon injury, migratory keratinocytes adapt their terminal differentiation program to the healing process. The response of activated keratinocytes is characterized by suppressed expression of differentiation-specific keratins K1 and K10, and de novo production of K6, K16 and K17, differing from keratins of the healthy epidermis (Freedberg et al., 2001; Hudson et al., 2009; Lessard et al., 2013; Usui et al., 2008). The expression of K6 and K16 occurs within 6 h, and K17 up to 12 h after injury (Paladini et al., 1996). The accumulation of these keratins at the wound edge corresponds to polarized reorganization of keratin filaments in suprabasal keratinocytes, when K1/K10 move from the cytoplasmic periphery and desmosomal binding sites into the perinuclear space, opposite to the direction of migration. These cytoskeletal changes provide sufficient plasticity that is required during migration (Moll et al., 2008).

The key initiators of K6 and K16 expression are pro-inflammatory signals IL-1 and TNF-α (Freedberg et al., 2001; Komine et al., 2001). In this context, K17 has been implicated in protection of keratinocytes against apoptosis induced by TNF-α, thus promoting their survival during inflammation (Suter et al., 2009; Tong and Coulombre, 2006). K16 is also involved in innate
immune responses by regulating important transmembrane Toll-like receptors responsible for various contaminants and damage-associated molecular patterns detection during inflammation (Wu et al., 2008, 2009).

The interleukin-1 family: endogenous mediators of the inflammatory response

Damaged keratinocytes release pre-stored cytokines, especially IL-1α, and the detection of endogenous danger signaling molecules, as well as the sensing of exogenous threats by membrane receptors, leads to NF-κB-mediated transcription of a plethora of newly induced enzymes, chemokines, antimicrobial peptides and pro-inflammatory cytokines (Suter et al., 2009). The majority of keratinocyte-derived cytokines operating in the skin are members of the IL-1 family.

The most important members of the IL-1 family involved in keratinocyte-mediated inflammatory responses are IL-1α, IL-1β, IL-18, and IL-33 that control acute inflammatory processes. The receptor antagonist IL-1Ra, constitutively expressed by keratinocytes, acts as a competitive inhibitor of IL-1α, therefore an increased ratio of IL-1Ra to IL-1α results in reduced activity of this cytokine (Uchi et al., 2000; Zepter et al., 1997). On the other hand, IL-1Ra deficiency may contribute to a prolonged inflammatory response through increased NF-κB activity, leading to prolonged production of pro-inflammatory cytokines (Behn et al., 2012; Ishida et al., 2006; Schreml et al., 2010).

The keratinocyte-derived IL-1α may act in a paracrine manner on the proliferation of nearby fibroblasts, production of collagen or pro-inflammatory cytokines such as IL-6, and expression of intercellular adhesion molecule (ICAM) on endothelial cells (Freedberg et al., 2001; Komine et al., 2001). IL-1α autocrine signaling in keratinocytes contributes to the production of cytokines IL-6, IL-8 and TNF-α (Komine et al., 2001). IL-1α also promotes conversion of a structural protein and induction of migration and proliferation of keratinocytes (Freedberg et al., 2001; Sivamani et al., 2007) (Fig. 1). Apart from IL-1α binding to its corresponding cell surface receptors, the precursor form of IL-1α can translocate to the nucleus and exhibit a dual functionality. The significance of nuclear function lies in the stimulation of pro-inflammatory gene transcription even if the surface IL-1R is completely blocked (Dinarello, 2011; Werman et al., 2004).

Interleukin-1α and interleukin-1β

The pro-inflammatory cytokine IL-1α and its close homologue IL-1β are identical in their actions (Wu et al., 2014). Although they are encoded by divergent genes (Luger and Schwarz, 1990), they operate through the same receptor. Keratinocytes initially synthesize both pro-IL-1α and pro-IL-1β as 31-kDa precursors. However, IL-1α is the predominant form (Busbridge et al., 1989). It is constitutively expressed and retained in the cytoplasm of keratinocytes to prevent binding to specific receptors (Coquette et al., 2003; Freedberg et al., 2001; Luger and Schwarz, 1990). Whereas maturation of IL-1β requires cleavage by the inflammasome-activated cytoplasmic protease caspase-1 into its biologically active 17-kDa form, IL-1α is active in its full-length form (Yazdi and Drexler, 2012). It has been generally accepted that the majority of IL-1α is passively released from dying cells as a result of harm. This theory was supported by the fact that IL-1α is synthesized without a signal peptide (Erdag and Morgan, 2002; Ishida et al., 2006). However, it has been shown that proteolytic processing of pro-IL-1α by the calcium dependent protease calpain causes a several times enhancement of biological activity (Afonina et al., 2015). Gross et al. (2012) hypothesized that the intracellular complex inflammasome could also be involved in activation of IL-1α in keratinocytes. According to their observations, secretion and processing of IL-1α occurs downstream of the inflammasome NLRP3, depending on the NLR agonist (Gross et al., 2012).

In response to IL-1 secretion, keratinocytes synthesize two types of IL-1 receptors; biologically active type I IL-1R and inactive type II that acts as a decoy receptor for IL-1 (Blanton et al., 1989). The receptor antagonist IL-1Ra, constitutively expressed by keratinocytes, acts as a competitive inhibitor of IL-1α, therefore an increased ratio of IL-1Ra to IL-1α results in reduced activity of this cytokine (Uchi et al., 2000; Zepter et al., 1997). On the other hand, IL-1Ra deficiency may contribute to a prolonged inflammatory response through increased NF-κB activity, leading to prolonged production of pro-inflammatory cytokines (Behn et al., 2012; Ishida et al., 2006; Schreml et al., 2010).

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![Fig. 1. Schematic signaling of keratinocyte-derived IL-1α.](image-url)

As a result of injury, damaged keratinocytes release pre-stored IL-1α. Binding of IL-1α to its receptor triggers inflammatory pathways responsible for production of pro-inflammatory cytokines (IL-6, TNF-α) and chemokines (IL-8), which attract immune cells (neutrophils) into the wound bed. Autocrine signaling of IL-1α also compensates for the death of keratinocytes by inducing their proliferation. IL-1α exerts its proliferative and pro-inflammatory effect on dermal cells (fibroblasts) as well.
Interleukin-18

The expression of this pro-inflammatory cytokine has been observed to be increased during the early phase of wound healing. The main producers of interleukin-18 (IL-18) during wound healing are keratinocytes at the wound margin (Kampfer et al., 1999). IL-18 has been identified as an inducer of cytokine and chemokine production, thus contributing to attraction of immune cells into the wound area (Dinarello, 1999; Kampfer et al., 1999). The keratinocytes synthesize an inactive pro-peptide IL-18 that requires proteolytic cleavage into its active mature form by inflammasome-activated caspase-1. Some publications indicate that human keratinocytes release only the unprocessed form of this cytokine (Companjen et al., 2000; Mee et al., 2000). Opposite results have been achieved by immunoblotting analysis, proving the cytoplasmic localization of IL-18 in living epidermal layers (Koizumi et al., 2001). Novel publications favor the ability of inflammasome-caspase-1 processed IL-18 to activate keratinocytes in paracrine as well as autocrine fashions (Lee et al., 2015b; Roth et al., 2012). IL-18 exerts its activity through binding to the IL-18R receptor complex by induction of nuclear translocation of NF-κB (Dinarello, 1999, 2002; Xiao, 2016). Keratinocytes also express IL-18R, therefore they might be affected by autocrine IL-18 signaling (Koizumi et al., 2001).

Interleukin-33

The newly discovered member of the IL-1 family, inflammatory cytokine IL-33, is constitutively produced by healthy keratinocytes, thus contributing to maintenance of the epidermal barrier. It is expressed in non-proliferating keratinocytes of the outer protective layer. In an injured epidermis, IL-33 is involved in pro-inflammatory responses where it acts as an endogenous warning signal, alarmin, passively released by necrotic cells, triggering acute local inflammation and tissue repairation (Afonina et al., 2015; Balato et al., 2014; Martin and Martin, 2016). The novel study shows undetectable expression of IL-33 during steady-state in the intact epidermis and robust induction during acute inflammation. This argued against a role of IL-33 as a pre-stored alarmin and favored its inflammation-induced novel synthesis (Sundnes et al., 2015). IL-33, similarly to IL-18 or IL-1β, is a protein without signal peptide and is secreted by a poorly characterized mechanism inside the endoplasmic reticulum of the Golgi bodies (unconventional pathway). Some authors propose that IL-33 is converted into its mature active form in the same way by caspase-1 (Keller et al., 2008; Weinheimer-Haus et al., 2015). However, this remains disputed (Luthi et al., 2009). The pro-inflammatory action of IL-33 is also controversial. Whereas detection of extracellular IL-33 results in activation of the NF-κB signaling pathway, its endogenous overexpression is associated with prevention of DNA-binding and transcriptional activity of NF-κB subunits (p65) (Ali et al., 2011).

Keratinocyte-derived pro-inflammatory mediators of inflammation

Tumor necrosis factor α

Tumor necrosis factor-α is a pro-inflammatory cytokine with a key role in regulating the inflammatory response. In healthy epidermis, TNF-α is only situated in the upper layers to a limited extent (Freedberg et al., 2001). Production of TNF-α by keratinocytes is increased under pathological conditions, such as chemical irritation, bacterial infection and UV irradiation (Freedberg et al., 2001; Weiss et al., 2004). Its expression is upregulated by IFN-γ or IL-1α (and vice versa) (Uchi et al., 2000).

TNF-α is synthesized as a membrane-bound precursor and its proteolytic activation by TNF-α converting enzyme TACE releases a soluble monomer that forms a biologically active homotrimer (Charles et al., 2009; Horiiuchi et al., 2010). TNF-α mediates its effect through engagement with two structurally divergent receptors, TNFR1 and TNFR2. Keratinocytes particularly express TNFR1 (Uchi et al., 2000).

Besides its action in cutaneous inflammation, TNF-α regulates cell migration by inducing changes in expression of transmembrane receptors (integrins), cytoskeletal rearrangements and the induction of matrix metalloproteinase-9 (MMP-9) (Banno et al., 2004).

Normally, TNF-α reaches its maximum level between 12 and 24 h and then decreases to its basal level. Under some circumstances, especially diabetes mellitus, enhanced and prolonged expression of TNF-α contributes to chronic inflammation and soft tissue damage (Banno et al., 2005; Xu et al., 2013). On the other hand, the complete inhibition of TNF-α is associated with increased risk of infections and default tissue repair. Targeted control of TNF-α expression is therefore required for proper wound healing (Trent and Kerdel, 2005).

Interleukin-6

Interleukin-6 is a pleiotropic cytokine exhibiting multiple effects, and is mainly involved in the host inflammatory responses (Sugawara et al., 2001). IL-6-induced pro-inflammatory action in keratinocytes leads to the production of inflammatory cytokines and chemokines as well as promotion of keratinocyte growth and proliferation (Erdag and Morgan, 2002; Grossman et al., 1989; Hanel et al., 2013; Saggini et al., 2014; Sawamura et al., 1998).

IL-6 is synthesized with a signal peptide and healthy keratinocytes produce and actively release very low levels of IL-6 (Sugawara et al., 2001). Disruption of the epidermal layer exerts an enhancing effect on IL-6 expression (Hanel et al., 2013). IL-1α released from injured keratinocytes is a stimulus for IL-6 gene expression induced by transcription factors NF-κB and NF-IL-6 (CCAAT/enhancer binding protein beta - C/EBPβ). Stimulated keratinocytes produce IL-6, especially at the leading edge of the wound.

However, the amounts of IL-6 are also increased under pathological conditions, such as chronic skin disease (Grossman et al., 1989; Saggini et al., 2014) or bacterial infection. A novel study reported that impairment of the important epidermal layer barrier, the stratum corneum, following Staphylococcus aureus treatment of an in vitro epidermal model was at least partially caused by S. aureus-induced IL-6 in keratinocytes. IL-6 is responsible for abnormal epidermal differentiation, leaving the skin vulnerable to invasion by pathogens (Son et al., 2014).

Interleukin-8

Keratinocytes stimulated by IL-1α, IL-1β, TNF-α, as well as ligands of TLR receptors, activate the NF-κB pathway resulting in accumulation of IL-8 (Su and Richmond, 2015; Wilmer and Luster, 1995). Keratinocytes are a major source of the pro-inflammatory cytokine IL-8, which has a direct effect on the initial inflammatory phase when acting as a chemokine (Grimstad et al., 2011).

IL-8 operates through binding to its receptor (IL-8R). Keratinocytes also express IL-8R in response to inflammatory stimuli and IL-8 can also act as a chemo-attractant to keratinocytes, inducing their migration along the gradient. Moreover, IL-8R is expressed to a lower extent in chronic wounds than in acute wounds. This fact suggests that a decrease in IL-8R levels causes an impairment in the normal wound healing process (Jiang et al., 2012). Devalaraja et al. (2000) demonstrated delayed wound healing in IL-8R-
deficient mice and suppression of the proliferative response in primary cultured keratinocytes in vitro.

**Interleukin-24**

In 1999, Soo et al. (1999) published a report describing overexpression of IL-24 during repair of excisional skin wounds. Expression of the IL-24 gene increased within 12 h for up to 5 days and then gradually decreased to baseline levels (Soo et al., 1999; Wang and Liang, 2005). Inducers of IL-24 production are the acute phase pro-inflammatory cytokines such as IL-1, IL-6, or TNF-α. However, chronic inflammation-associated cytokines IL-4, IL-17A, and IL-22 can also up-regulate expression of IL-24 in keratinocytes. Therefore, the overproduction of IL-24 during inflammation seems to be detrimental to wound healing (Jin et al., 2014). A study comparing human chronic and early acute wounds showed higher levels of expression of IL-24 and its receptor in chronic wounds (Bosanquet et al., 2012). IL-24 has also been reported to diminish the proliferative and migratory phenotype of keratinocytes (Bosanquet et al., 2012; Poindexter et al., 2010). Another study even ascribes a keratinocyte-derived IL-24 role in the positive self-amplifying cycle of epidermal inflammation by its ability to increase keratinocyte production of IL-8, prostaglandin E2 (PGE2) and matrix metalloproteinase-1 (MMP1) (Jin et al., 2014).

**Cyclooxygenase (COX) enzymes**

Keratinocytes express two isoforms of cyclooxygenase (COX), referred to as COX-1 and COX-2. COX-1 is produced constitutively in normal and wounded skin to the same extent. The expression of COX-2 is rapidly induced in response to injury, inflammation and various agonists such as cytokines and growth factors, whereas in unwounded skin, the level of COX-2 is only slightly above the limit of detection. Its activation results in the synthesis of PGE2, which is a major endogenous product of arachidonic acid conversion in non-confluent keratinocytes in culture or intact skin during tissue healing (Fairweather et al., 2015; Konger et al., 1998; Muller-Decker et al., 2002). The levels of both COX-1 and COX-2 have been shown to be associated with non-healing wounds (Kampefer et al., 2003; Kendall and Nicolaou, 2013)

COX-2 stimulates proliferative activity in keratinocytes through PGE2, which acts as a mitogen for keratinocyte growth (Konger et al., 1998). According to this, inhibition of COX-2 could lead to delayed wound healing. Indeed, non-selective anti-inflammatory drugs (NSAIDs) controlling the production of PGE2 and PGD2 through blocking of the upstream enzyme COX, induce prolongation of the healing period of the cutaneous barrier that was disrupted by mechanical scratching (Honma et al., 2005; Sivamani et al., 2007). Conversely, a study published in 2002 has revealed no significant effect of oral administration of COX-1/COX-2 selective inhibitors on wound healing of full-thickness incisions in mouse skin (Muller-Decker et al., 2002). Another study on mice has explored keratinocyte responses to a selective COX-2 inhibitor during the healing of an abrasive injury and has confirmed no effect on proliferation or differentiation (Hardy et al., 2003). This is in contrast to recent research findings, when a COX-2 inhibitor, orally administered to mice with full-thickness wounds, significantly delayed wound closure (Fairweather et al., 2015).

**Reactive oxygen species (ROS)**

ROS are primarily thought to have detrimental effects on health. ROS are able to convert originally stable biological molecules into reactive radicals and can induce alterations in the structure of extracellular matrix proteins and lipids, degradation of tissue and DNA or disruption of membrane or enzyme functions (Wolfe et al., 2014).

Keratinocytes express membrane-bound NADPH oxidase, which produces superoxide anions as a defense against infectious pathogens (Wolfe et al., 2014). ROS generated by NADPH oxidase support the TNF-α-initiated activation of NF-κB (Wagener et al., 2013). Conversely, keratinocytes have been found to produce ROS through an NF-κB-signaling transduction pathway following stimulation with the inflammatory cytokine TNF-α (Reynaert et al., 2006; Young et al., 2008). Thus ROS can contribute to a significant enhancement of the inflammatory response. In this context, ROS have been recently reported to support the assembly of the immune protein complex NLRP3 inflammasome that is required for maturation of pro-inflammatory cytokines such as IL-1β or IL-18 (Corsini et al., 2013; Tschopp and Schroder, 2010; Wagener et al., 2013).

Some publications indicate detrimental effects of ROS on tissue by intensifying the inflammatory response following injury, which results in exacerbation of skin disease (Wagener et al., 2013; Young et al., 2008; Zhang et al., 2009). On the other hand, ROS are indispensable for endogenous protection against infectious pathogens, activation or deactivation of enzymes and various signaling pathways (Wagener et al., 2013).

A study of wound healing effects of ROS induced by lipopolysaccharide (LPS) demonstrated a dose-dependent response. Although protective epithelial responses have been activated by non-toxic LPS concentrations via the generation of ROS, and wound repair was accelerated, an LPS concentration exceeding the threshold led to a slowdown in healing and potential epithelial damage. It has been proposed that the LPS-induced stimulation of wound regeneration in the airway epithelium occurs via cooperation of TLR4 and NADPH oxidase (Koff et al., 2006). This interaction also leads to ROS formation and activation of redox-sensitive transcription factor NF-κB (Koff et al., 2006; Sadikot et al., 2004).

Therefore, cellular antioxidant defense systems are necessary for maintaining the redox equilibrium. Normal wound healing is characterized by balanced neutralization of ROS toxicity by ROS-detoxifying molecules, known as antioxidants. The overproduction of ROS in the case of depletion of antioxidants leads to detrimental oxidative stress and chronic inflammation (Behm et al., 2012; Wagener et al., 2013).

**Toll-like receptors mediate the early inflammatory response**

TLRs are transmembrane glycoproteins expressed by cells of the innate immune system or non-immune cells such as keratinocytes. Keratinocytes express TLR1–6, TLR9 and to a lesser extent TLR10 (Chen et al., 2013; Lee et al., 2010). Proliferating basal keratinocytes express mainly TLR5, whereas differentiated suprabasal layers express TLR1, TLR2 and TLR9. TLR4 is present throughout the epidermis (Miller et al., 2005; Portou et al., 2015).

In the event of a skin injury, induced expression of cytokines, chemokines as well as cell adhesion molecules is triggered in keratinocytes by TLR2, TLR3, TLR4, TLR5 and TLR9 signaling (Chen et al., 2013; Lee et al., 2010). TLRs are involved in detection of microbial pathogens and self-originated signaling molecules that are released by damaged cells, through their transmembrane domain and a cytoplasmic domain with a TIR region that is shared by the IL-1 receptor (Miller and Modlin, 2007). TLRs and IL-1R activations are quite similar. Both of them share the adaptor protein myeloid differentiation factor-88 (MyD88) and promote pro-inflammatory signaling cascades (Hari et al., 2010). Signal transduction from these receptors converge on the nuclear translocation of transcription factor NF-κB and activation of JNK/p38 MAPK pathways (Baker et al., 2003; Chen et al., 2013).
Considerable attention has been paid to activation of TLR4 in wound healing. TLR4 recognizes exogenous LPS, especially from Gram-negative bacteria, as well as endogenous early mediators of inflammation and injury (DAMPs), including the small nuclear binding protein HMGB1 (Mollen et al., 2006; Srikrishna and Freeze, 2009) or antimicrobial peptides released by keratinocytes (Nestle et al., 2009). TLR4 are particularly expressed in keratinocytes at the wound edges. Chen et al. (2013) demonstrated a disturbed wound closure and delayed re-epithelialization in TLR4-deficient mice, together with changes in the levels of IL-1β, IL-6 and epidermal growth factor (EGF) as a result of the reduction of proliferating keratinocytes in the wound. Similarly, Eslami et al. (2014) demonstrated an acceleration of re-epithelialization in vitro with a wound scratch assay, as well as a trans-well migration assay, performed on human corneal epithelial cells. An experiment in vivo in a murine corneal epithelial injury model performed by the same authors revealed peak expression of TLR4 at 6 h with a return to baseline in 18 h, indicating that epidermal injury induces early expression of TLR4 (Eslami et al., 2014). Prolonged expression of TLR4, on the contrary, leads to delayed inflammation and impaired wound healing, as seen in chronic venous ulcer patients (Pukstad et al., 2010).

Other TLRs may alternate with TLR4 in identification of endogenous injury signaling molecules. For example, detection of non-coding double-stranded RNA from necrotic keratinocytes by TLR3, or HMGB1 by TLR2, has been observed (Borkowski et al., 2013; Mollen et al., 2006). Moreover, TLR4 deficiency results in significantly increased TLR2 expression, suggesting a compensatory role of TLR2 (Mollen et al., 2006). Conversely, increased expression of cell surface TLR2 or TLR4 contributes to excessive NF-κB activity and an imbalance in the inflammatory production of mediators, leading to obstruction of wound healing (Dasu and Martin, 2014; Dasu et al., 2010).

Participation of various TLRs in the early phase of wound healing has already been experimentally proven (Chen et al., 2013; Dasu and Isseroff, 2012; Kishibe et al., 2015; Mollen et al., 2006; Sugahara et al., 2014), but controversy exists as to whether the effect is beneficial or detrimental (Dasu and Isseroff, 2012). Thagia et al. (2015) published a detrimental effect of TLR5 signaling pathways on wound healing via enhancement of TNF-α mRNA, compared with other TLRs. On the other hand, the study of Borkowski et al. (2013) indicates a wound healing potential of TLR3, based on expression of genes critical for epithelial barrier restoration (ceramides, etc.) and inflammation associated with TLR3 activation in keratinocytes. Similarly, Sato et al. (2010) observed accelerated wound closure of excisional mouse wounds treated with TLR9 ligands as compared to untreated mice. Apparently, enhancement of intracellular TLR3 and TLR9 activation by their agonists improves wound healing, while the persistent or, conversely, deficient activation of TLR2/TLR4 on the cell surface delays the inflammatory phase and wound healing (Dasu and Isseroff, 2012; Pukstad et al., 2010).

TLRs are the most important subset of the pattern recognition receptor (PRR) family, which includes NOD-like receptors (NLR) (Portou et al., 2015). The NLRs are the cytosolic counterparts of TLRs, and thus function in the intracellular recognition of danger and represent a key component of the inflammasome (Sutterwala et al., 2014).

Typically, this multi-protein complex is made up of several components, including immune sensor proteins of the human NLR family, the cysteine-dependent protease caspase-1 and, in most cases, an apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) (Santana et al., 2016). Depending on the dangerous stimuli, the individual components of the relevant inflammasome are assembled in the cytosol to create a fully functional entity (Sutterwala et al., 2014). The stress-activated NLR protein recruits an adaptor protein, which is responsible for activation of zymogen procaspase-1 via multimerization, followed by autoproteolysis (Gross et al., 2012). The active caspase-1, in turn, controls maturation and secretion of proinflammatory cytokines IL-1β, IL-18 (Sollberger et al., 2014) and most likely IL-33 (Keller et al., 2008; Ogura et al., 2006). Thus, inflammasomes contribute to the host defense via tissue damage detection and participation in pathogen clearance during infection and injury (Schröder and Tschopp, 2010).

It has been previously assumed that expression of inflammasomes is limited to professional immune cells like macrophages. Therefore, most studies dealing with activation of these innate immune complexes are performed in these cell types. However, an interest has recently grown in the role of inflammasomes in non-myeloid cells such as epithelial cells or fibroblasts (Cassell et al., 2009; Zhang et al., 2015). Keratinocytes actively express the NLRP3 inflammasome, also known as NALP3 (Santana et al., 2016).

NLRP3 activation to form a functional complex in keratinocytes is promoted by a wide range of stimuli. Keratinocytes have been shown to mediate NLRP3 activity in response to various exogenous stressors, such as microbial infection or environmental irritants e.g. aluminum salts (Eisenbarth et al., 2008) and UV light (Feldmeyer et al., 2007; Santana et al., 2016) or chemicals (Watanabe et al., 2007) and host-derived molecules released from infected cells, including extracellular ATP (Santana et al., 2016). Most of these stimuli induce excessive ROS production, which triggers transcriptional activation of NF-κB or the p38 MAPK signaling pathway, and is involved in induction of NLRP3 assembly (Corsini et al., 2013). ROS could also increase the intracellular Ca2+ concentration (Feldmeyer et al., 2007; Rimessi et al., 2015) or lysosomal cathepsin from damaged lysosomes in yet unknown ways, leading to activation of the inflammasome and secretion of its downstream cytokines (Brandstetter et al., 2015). A novel study also reported that a bacterial toxin, amylosin, caused a dose-dependent potassium ion efflux and depolarization of mitochondria in keratinocytes (Rasimius-Sahari et al., 2015), which are known to activate the NLRP3 inflammasome in macrophages (Munoz-Planillo et al., 2013). Another study indicates an important role of the T-cell-derived cytokine IFN-γ (Niebuhr et al., 2014). IL-17 and IL-22 in activation of NLRP3 via induced expression of TLR receptors in keratinocytes under inflammatory conditions (Cho et al., 2012).

In general, NLRP3 inflammasomes require a priming step before activation. Events leading to increased cellular expression of NLRP3 inflammasome components are ligand binding to TLRs (especially LPS recognition by TLR4), and cytokines binding to IL-1R or tumor necrosis factor receptor 1 (TNFR1), which results in NF-κB stimulation (Guo et al., 2015; Sutterwala et al., 2014). However, keratinocytes are thought to constitutively express inflammasomes proteins and the pro-inflammatory cytokines pro-IL-1β or pro-IL-18. According to some authors, in contrast to macrophages, activation of the NLRP3 inflammasome is independent of its previous induced expression (Sollberger et al., 2014). On the other hand, recent studies suggest that basal expression of inflammasomes in tissue homeostasis is not sufficient for activation during infection (Latz et al., 2013; Tervaniemi et al., 2016). Indeed, novel studies in vitro using Barrett’s epithelial cells or retinal pigment epithelial cells reported...
of NF-κB priming of an inflammatory and induced expression of inflammatory substrates such as pro-IL-1β or pro-IL-18 under inflammatory conditions (Higashimori et al., 2016; Liu et al., 2014). In this context, a novel study by Lee et al. (2015a) revealed an NF-κB role in increased caspase-1 expression and a significant NF-κB/caspase-1 axis for secretion of active IL-1α cytokine during the inflammatory phase of wound healing in the epidermis. Similarly, Watanabe et al. (2007) revealed upregulation of pro-IL-1β expression in primary keratinocytes by agonists of TLR receptors as well as TNF-α, resulting in activation of the NF-κB pathway. However, there was no effect on expression levels of pro-IL-18 (Watanabe et al., 2007). In contrast, IL-1α, similarly to IL-1β, IL-18 or IL-33, can give a positive feedback to activation of NF-κB, which initiates the expression of target genes such as IL-6 and IL-8 (Lee et al., 2015a; Wagener et al., 2013; Weber et al., 2010).

An in-depth understanding of activation of inflammases in keratinocytes could help to avoid hyperactivation of this molecule, resulting in excessive inflammation (Sutterwala et al., 2014). Autoinflammatory disorders are accompanied by mutations in the NLRP3 gene, which results in a constitutively active form of NLRP3 and uncontrolled activation of pro-IL-1β and pro-IL-18 (Cassel et al., 2009); dermatitis may arise following exposure to an allergen or sensitiser (Dai et al., 2011; Watanabe et al., 2007). Accordingly, blockage of inflammase NLRP3 in diabetic mice improved impaired wound healing that was characterized by a persistent inflammatory response (Bitto et al., 2014). On the other hand, the important role of the NLRP3 inflammase during early phases of wound healing has been recently described. Cutaneous wound healing in mice deficient in NLRP3 and caspase-1 revealed impaired wound healing, compared to wild type controls. This study suggests the importance of NLRP3 in efficient tissue repair, especially for early events of wound healing (Weinheimer-Haus et al., 2015).

The central role of NF-κB in inflammation

Transcription factor NF-κB has a wide range of actions. It is involved in the expression of genes coding for pro-inflammatory cytokines, chemokines, adhesion molecules and proteinases. In general, NF-κB controls cell differentiation and proliferation, and protects cells from apoptosis by expression of anti-apoptotic proteins. It can be induced by several stimuli e.g. inflammatory cytokines, bacterial and viral infection, UV light (Pasparakis, 2009).

In the intact epidermis, a cytosolic localization of NF-κB has been reported in all layers, although a nuclear site is only in suprabasal layers. This fact suggests a role for NF-κB in the switch from the proliferation to epidermal differentiation (Seitz et al., 1998). Therefore, overexpression of NF-κB has an anti-proliferative effect in keratinocytes, as opposed to dermal skin cells fibroblasts (Charles et al., 2009; Uchi et al., 2000).

In healthy skin, NF-κB is retained in the cytoplasm, in its inactive form, by binding to its IκB inhibitor family of proteins. IκB proteins form a complex with p50/p65 (NF-κB heterodimer), and thereby mask a nuclear localization of NF-κB. Following stimulation, the IκB kinase (IKK) complex phosphorylates IκB proteins, and thus induces their ubiquitination and proteasomal degradation that is required for the nuclear translocation and transcriptional function of NF-κB (Ashcroft et al., 2012). The activation of NF-κB is initiated via two distinct signaling pathways. The canonical pathway is triggered by signals from cytokine receptors such as TNFR1, IL-1R and PRR (especially TLR4) and generally leads to nuclear translocation of p65-containing heterodimers. The minor non-cannonical pathway is activated by specific members of the TNF cytokine family and leads to processing of precursor p100 with subsequent formation of a p52/ReIB complex. This alternative pathway plays an important role in the immune system (Oeckinghaus et al., 2011).

Within acute inflammation, keratinocytes respond, especially with the canonical NF-κB pathway, by various mechanisms including pro-inflammatory molecules such as cytokines (IL-6, IL-1, TNF-α) and chemokines (IL-8) or production of inducible enzymes (Fig. 2). In addition, the canonical NF-κB pathway supports cell survival and proliferation (Ambrozova et al., 2017; Etemadi et al., 2015; Kumari et al., 2014).

Stimulation of the TNFR1 signaling pathway leads to recruitment of TNF receptor-associated factor 2 (TRAF2) adaptor proteins (Oeckinghaus et al., 2011) which finally result in activation of canonical NF-κB signaling. Since NF-κB is responsible for the production of a variety of anti-apoptotic genes (Etemadi et al., 2015), NF-κB-deficient cells are sensitive to TNF-α-induced apoptosis (Karin and Lin, 2002). Under normal conditions, once microbial and bacterial contamination in the wound is eliminated, TNF signaling is switched off. It has recently been reported that a deficiency in TRAF2 in keratinocytes activates the constitutive non-canonical pathway with subsequent induction of apoptotic cell death and sustained production of pro-inflammatory cytokines (including TNF-α) and chemokines without the need for pathogenic stimulation. This self-sustained inflammation could be triggered by endogenous danger signals released from TNF-dependent death cells, which attract neutrophils and induce inflammation (Etemadi et al., 2015). Therefore, disrupted TNF-mediated NF-κB signaling might be detrimental to the resolution of inflammation. On the other hand, controlled apoptosis is essential to prevent excessive inflammation, and is important in the elimination of infected and damaged cells and maintenance of tissue homeostasis (Sollberger et al., 2014). Thus, canonical NF-κB signaling exerts two diverse roles, triggering inflammation while, at the same time, protecting against apoptotic effects arising from TNF-α binding to TNFR1 (Tak and Firestein, 2001; Wullaert et al., 2011).

Although inhibition of NF-κB seems to be an appropriate therapeutic target due to its strong pro-inflammatory potential, NF-κB silencing impairs wound healing in an epidermal scratch assay in vitro, as has been reported by Melchionna et al. (2012). The stimulatory environment is a decisive factor responsible for protective or detrimental actions of NF-κB. Skin wounding facilitates NF-κB-driven proliferation and migration of keratinocytes (Ambrozova et al., 2017). Therefore, NF-κB inhibition impairs re-epithelialization, an essential component of wound closure (Melchionna et al., 2012).

Keratinocytes are constantly challenged by various microbial or environmental attacks. NF-κB represents a crucial regulator of immune homeostasis in the epidermis, integrating inflammatory and immune circuits in order to maintain a well-controlled response to infection and injury (Ambrozova et al., 2017; Wullaert et al., 2011).

Conclusion

This review highlights the essential role of keratinocytes during inflammation caused by disruption of an intact epidermal barrier and pathogen invasion. The injured keratinocytes change their scheduled task from being a stable mechanical/antimicrobial barrier to actively responding to inflammation by transformation of cytoskeletal proteins and activation of corresponding inflammatory pathways. To ensure an efficient defense against infection, the keratinocytes produce pro-inflammatory cytokines and chemokines resulting in recruitment of effector immune cells. An unregulated inflammatory response, accompanied by unmanageable and delayed production of pro-inflammatory mediators, results in T-cell infiltration and death of inflammatory cells. These
conditions generate a chronic inflammatory state, negatively affecting skin homeostasis. This review summarizes our current understanding of inflammatory events orchestrated by keratinocytes and examines their positive or negative effects on wound healing. However, conflicting results and conclusions demonstrate that inflammation is a complex, coordinately regulated process that requires further in-depth investigation.

Conflict of interests

The authors declare that they have no conflict of interest.

Authorship contribution

JJ: writing of the manuscript; JF: critical revision of the manuscript; JU: critical revision and final approval of the version to be published.

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