Innate and Adaptive Immune Responses in Wound Epithelialization

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Abbreviations and Acronyms

AIM2 = absent in melanoma 2
AMPs = antimicrobial peptides
CAR = coxsackie and adenovirus receptor
CW = chronic wound
DAMPs = damage-associated molecular patterns
DETC = dendritic epidermal T cells
DFU = diabetic foot ulcer
FGF = fibroblast growth factor
hBDs = human β-defensins
HMGB1 = high-mobility group box 1
HSPs = heat shock proteins
IFN-1 = interferon type 1
IGF = insulin growth factor

(continued)

Significance: Over the years, it has become clear that, in addition to performing their regular duties in immune defense, the innate and adaptive arms of the immune system are important regulators of the complex series of events that lead to wound healing. Immune cells modulate wound healing by promoting cellular cross-talk; they secrete signaling molecules, including cytokines, chemokines, and growth factors. In line with the major effort in wound healing research to find efficient therapeutic agents for the constantly increasing number of patients with chronic wounds, findings regarding the contributions of innate and adaptive immune responses to the re-epithelialization of damaged skin may bring novel therapeutics.

Recent Advances: Increasing evidence suggests that induction of the adaptive immune response requires activation of innate immunity and that there is a dependent relationship between the two systems. Consequently, the bridge between the innate and the acquired immune systems has become an area of emerging exploration. It is clear that a better understanding of the epithelial cells (keratinocytes), immune cells, and mechanisms that contribute to an effective wound healing process is necessary so that new strategies for successful wounds treatment can be devised.

Critical Issues: A greater understanding of the biology of skin innate and adaptive immune cells during wound epithelialization may have an impact on development of novel strategies for significant improvements in the quality of tissue repair.

Future Directions: Future studies should clarify the importance of particular molecules and mechanisms utilized for development and functions of skin-resident $\gamma\delta$T and Langerhans cells, as well as identify therapeutic targets for manipulation of these cells to combat epithelial diseases.

SCOPE AND SIGNIFICANCE

WOUND HEALING IS a well-coordinated process with the ultimate goal of barrier restoration. Acute wound healing consists of a highly orchestrated series of events and is regulated by a variety of cells, including immune cells. Immune cells modulate wound healing by promoting cellular cross-talk via secreting signaling molecules, including cytokines, chemokines, and growth factors. For successful wound healing, a delicate balance in this process needs to be maintained. However, an environment with excessive cytokine and protease production can lead to persistent inflammation and consequent impaired wound healing. The focus of this review is on understanding how elements of the immune

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system contribute to the re-epithelialization of damaged skin.

TRANSLATIONAL RELEVANCE

An impaired immune system has been demonstrated to severely impede the wound healing process. Although current knowledge allows us to recognize the complexities of various cell types involved in skin defense mechanisms and wound immunology, specific roles and interactions between keratinocytes and other immune cells are poorly understood. Despite our understanding of immunity during acute wound healing, little is known about the integrity of defense mechanisms in patients with nonhealing CWs.

CLINICAL RELEVANCE

The management of chronic wounds (CWs) is an enormous clinical and economical burden on the healthcare system. In addition to the economic impact, the morbidity and mortality associated with CWs are considerable. Better understanding of the immune system at the cellular and molecular level within the CW environment, where the battle between microbes and host immune system is taking place, would possibly lead to new diagnostic techniques helping clinicians make appropriate treatment decisions, potential markers that would predict healing outcome, and advanced new therapeutic modalities.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

Overview of the immune system

The immune system is a constellation of cellular interactions functioning to protect the host from outside harm. A critical role of this system is to discern harmful pathogens from the host's own healthy tissues. While generating a sufficient response to maximally eliminate pathogens, the defense mechanisms must skillfully avoid damaging self tissues allowing for proper wound healing.

Immunity consists of two components: innate and adaptive (Fig. 1). Specialized immune cells that reside in discrete organs (*e.g.*, spleen, lymph nodes, skin, *etc.*) function to protect the body against various invaders. Although the innate and adaptive responses are distinct, growing evidence supports interplay between the two branches. The innate response represents immediate action, while the adaptive response is considered to take a later action. Each component can influence the magnitude and type of response of its counterpart. Moreover, it has been well described that certain cell types can exhibit several characteristics that place them at the border between the innate and the adaptive immune system.

Innate immunity

Pattern recognition receptors and toll-like receptors. The innate immune system is considered the first line of host defense. This is important for a proper response to injury, and its rapidity is crucial in eliminating the spread of infection. The nonspecific reaction relies on recognition of highly conserved structures of microorganisms, pathogen-associated molecular patterns (PAMPs), or damage-associated molecular patterns (DAMPs), by a class of receptors called pattern recognition receptors (PRRs).[1] The known PAMPs include fundamental structures for the survival of microorganisms (*e.g.*, bacterial lipopolysaccharide, lipopeptides, peptidoglycan, bacterial DNA, and double-stranded RNA),[1] while known DAMPs are mostly cytosolic and nuclear proteins (*e.g.*, chromatin-associated protein high-mobility group box 1 [HMGB1], heat shock proteins [HSPs], DNA, S100 molecules, and purine metabolites

Abbreviations and Acronyms *(continued)*

IL = interleukin

JAML = junctional adhesion molecule-like protein

KGF = keratinocyte growth factor

LCs = langerhans cells

LL-37/hCAP18 = cathelicidin

MAPK = mitogen-activated protein kinase

MHC = major histocompatibility complex

NLRs = NOD-like receptors

PAMPs = pathogen-associated molecular patterns

pDC = plasmacytoid dendritic cells

PRRs = pattern recognition receptors

TCR = T-cell antigen recognition receptor

Th1 = T helper type 1

TLR = toll-like receptor

VU = venous ulcer

$\gamma\delta$T cells = gamma delta (+) T lymphocytes

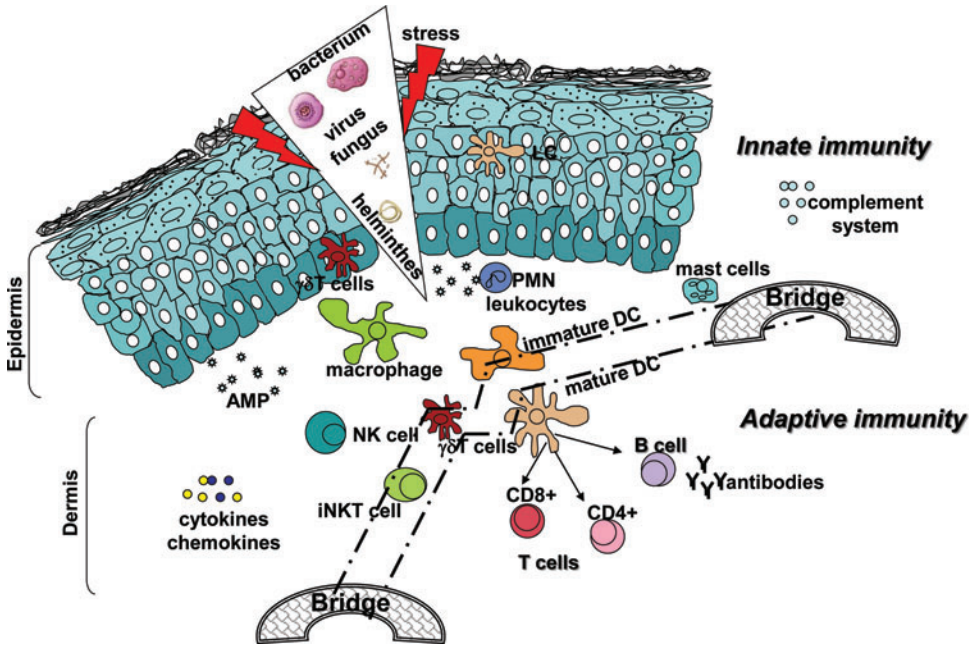

Figure 1. Schematic representation of the general innate and adaptive immune responses in the epidermis and dermis. Upon infection with a pathogen the cells of the innate immune response offer immediate, but short-lasting help. This leads to DC, γδT cell, and iNKT and NK cells activation, which forms the bridge between the innate and adaptive immunity. The cells of the adaptive immune response provide pathogen-specific, long-lasting protection for a proper wound healing process to take place. NK, natural killer. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

released during cellular stress, inflammation, or necrotic cell death. A recent study showed that HMGB1 has chemotactic effects on keratinocytes and fibroblasts *in vitro* and enhances diabetic wounds epithelialization *in vivo* upon topical application.[2]

PRRs are constitutively expressed on professional antigen-presenting cells and on nonprofessional immune cells.[1,3] Four different classes of PRRs have been identified including two transmembrane proteins, toll-like receptors (TLRs), and C-type lectin receptors; and two cytosolic proteins, retinoic acid-inducible gene-I-like receptors, and NOD-like receptors (NLRs).[3] This limited range of antigen recognition receptors allows for an immediate immune response. The most well characterized PRR is the TLR. Distinct TLRs recognize PAMPs and/or DAMPs. The activation of TLRs by their corresponding ligands leads to the subsequent activation of the NF-kB and mitogen-activated protein kinase (MAPK) pathways through adaptor proteins including MyD88, TIRAP/Mal, TRIF, and TRAM.[4] Cytokines, including interleukins (IL); IL-1, IL-6, IL-8, IL-12, and tumor necrosis factor alpha are then produced, stimulating neighboring cells to secrete chemokines. Inflammatory cells thus migrate to the stressed site to assist the innate immune response.[4]

The secretion of inflammatory mediators via TLR-dependent activation also promotes maturation of dendritic cells.[5] Mature dendritic cells are then able to prime the adaptive immune response by inducing T cell maturation and T helper type 1 (Th1) polarization.

The effects of TLR activation on wound healing have been suggested to be dose dependent. Higher concentrations may have growth-suppressive effects, while lower concentrations are suggested to promote regeneration.[6] Moreover, adenosine, an $A_{2A}R$ agonist, accelerated wound closure in $MyD88^{+/+}$ mice, while treatment in $MyD88^{-/-}$ mice had minimal effect. $A_{2A}R$ agonists and TLR agonists have a synergistic regulatory effect on macrophage phenotype and, given these findings, have been speculated to have a role in mediating wound repair.[6] Furthermore, topical application of immunostimulatory CpG oligodeoxynucleotoides promoted wound closure in mice by accelerating re-epithelialization and granulation tissue formation via TLR9-dependent mechanisms.[7]

A potent PAMP, lipopolysaccharide, inhibited keratinocyte migration in a dose-dependent manner and this inhibition was relieved using neutralizing antibodies to TLR4 and TLR2.[8] Recently, TLR4 was reported to be present in keratinocytes at the wound edges and epithelialization was

significantly delayed in TLR4-deficient mice.[9] Moreover, increased TLR2 expression has been shown to contribute to prolonged inflammation and delayed wound healing in the experimental diabetic wounds.[10] In this regard, wound fluid was collected from nonhealing venous ulcers (VU), demonstrating persistent TLR2 and TLR4-stimulating activities, whereas in healing wounds, they decreased over time, further supporting a role of elevated TLRs in the CW healing outcome.[10]

Keratinocytes role in innate immunity. Epithelia, the body's first-line defense barrier, significantly contribute to innate immunity. The skin is often regarded for its passive role as a mechanical barrier in innate immunity; yet, increasing evidence suggests that cells within the epidermis have an active role in initiating host defense.

Along with keratinocyte structural support properties, recent literature explores the mechanisms related to their innate immune functions, for example, promotion of resistance to skin infections and a regulation of inflammation. The protection conferred by keratinocytes is largely due to its production of antimicrobial peptides (AMPs). Two well-studied families of keratinocyte-secreted AMPs are human β-defensins (hBDs) and human cathelicidin (hCAP18/LL-37). Keratinocytes express AMPs either constitutively (hBD-1) or during inflammation (LL-37 and hBD-2, -3; Table 1).[11–15] Each of the AMPs possesses cytotoxic properties. LL-37, identified in humans, is markedly expressed in keratinocytes of inflammatory skin conditions.

In addition to their direct cytotoxic effects, hBDs and LL-37 are chemotactic agents for inflammatory cells.[16] In keratinocytes, LL-37 induces expression of proinflammatory cytokines and leads to chemotaxis of adaptive immune cells. Furthermore, hBDs can stimulate mast cell degranulation and keratinocyte production of cytokines and chemokines during acute inflammation.[16]

In the context of wound closure and immunity, recent data suggest the association between epithelial regeneration and AMP production. HBDs were shown to induce keratinocyte proliferation and migration, while hCAP18 production was induced in human wound edge keratinocytes.[16] Conversely, CW demonstrate lower levels of LL-37 expression.[16] Interestingly, LL-37 protein levels were low in CWs, while mRNA expression levels were elevated. Based on these results, Heilborn *et al.* suggest that the lack of AMP production in nonhealing wounds may lie in the post-translational processing.[17] Furthermore, α-defensins were found to be expressed in the epidermis of pressure ulcers and were proposed to be a possible indicator of a wound healing status.[18] Data regarding hBD2 expression in CWs are perplexing. While one study reports HBD2 induction in the epidermis after acute injury and constitutive expression in VU,[16] another study reports the same expression in CWs as in normal skin.[19] The latest study confirmed induced expression of hBD-2, absence of LL-37, and heterogeneous expression of hBD-3 in VU.[20] Observed differences can be accounted for as variations in tissue sampling from human CWs, a frequent difficulty experienced in translational studies. Taken together, the deregulated expression of AMPs in CWs highlights the likely dependence of proper wound repair on AMPs to generate an appropriate host response.

Along with producing AMPs, keratinocytes express TLRs. TLR signaling results in the production of cytokines and chemokines thus mediating the recruitment of effector immune cells.[4] Different TLRs are shown to be expressed throughout normal human epidermis (Fig. 2).[21–23] A recent study showed TLR4 localization in keratinocytes at the wound edge indicating its essential role in early skin wound healing.[9]

Table 1. *Expression of antimicrobial peptides in healthy human epidermis*

AMP	*Expression in healthy human epidermis*	*Reference*
LL-37	Upper epidermis of normal human skin.	12,13
hBD-1	Expression localized to the stratum basale and spinosum and/or stratum corneum.	13,14
hBD-2	Upper epidermis of normal human skin. Spinous and granular layers of epidermis.	13–15
hBD-3	Upper epidermis of normal human skin.	13

AMP, antimicrobial peptide.

Adaptive immunity

In contrast to innate immunity, the adaptive immune system provides a more delayed and specific response. The diverse repertoire of antigen receptors is generated from somatic gene rearrangements. The adaptive immune response is also unique in its ability to generate and retain memory; thus, it has the capability of providing a more rapid response in the event of subsequent immunologic challenge.

Adaptive immunity consists of humoral and cell-mediated responses, carried out by clonally distributed B and T cells. These effector cells are activated upon recognizing either free antigen or bound antigen via professional antigen presenting cells. For T cells, the type of effector response depends on the major histocompatibility complex

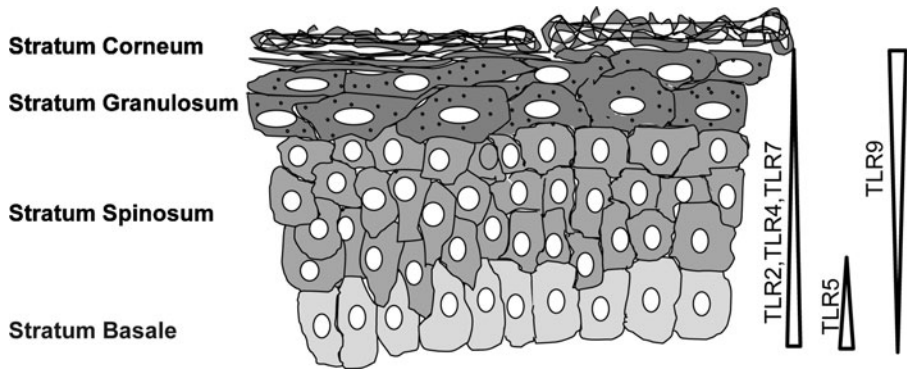

Figure 2. Toll-like receptor (TLR) expression in healthy human epidermis. Different studies report diverse expression of TLRs in healthy human epidermis. TLR2 and TLR7 are reported to be diffusely expressed throughout the epidermis with TLR 2 being more pronounced in suprabasal layers of epidermis.[21,22] TLR3 is barely detected in normal human skin.[22] TLR4 is reported to be diffusely expressed with a predominance beneath granular layer.[21,22] TLR5 is reported to be mostly present in basal epidermal keratinocytes.[23] TLR9 expression is weak in stratum basale and stratum spinosum but it is more expressed in upper, more differentiated keratinocytes.[23]

(MHC) molecule that is employed for antigen presentation. MHC class I complexes with endogenously produced antigens is recognized by CD8 cytotoxic T cells, while MHC class II expresses ingested exogenous antigen and is recognized by CD4 helper T lymphocytes. CD4 activation results in cytokine production and subsequent paracrine activation of neighboring cells, whereas CD8 cells are much more specific in cell targeting.

Activated B cells produce antibodies, which serve to inactivate toxins, opsonize bacteria, flag pathogens for destruction, and activate complement among other functions. Five different antibody classes exist: IgM, IgG, IgE, IgA, and IgD. Increasing evidence supports additional B-cell functions, including antigen presentation, cytokine production, and suppressive activity via IL-10 secretion.[24] Although distinct, T and B cells are highly interdependent. B cells serve as antigen-presenting cells for a primed specific T cell, while T cells produce cytokines that support B cell division and differentiation into the mature, antibody-producing plasma cell.[25]

It is likely that various subsets of T cells influence the wound environment differently since they secrete different cytokines. A series of experimental studies have indicated a role for T lymphocytes in wound healing as growth factor-producing cells and immunological effector cells.[26] Furthermore, there is evidence of direct cell–cell interactions of CD40 ligand expressing T lymphocytes with keratinocytes and their influence on the healing response.[27] A prolonged and increased presence of T cells and a nonstandard CD4-CD8 ratio, reported in chronic venous and diabetic foot ulcers (DFUs),[28] may be related to impaired epithelialization. However, very little has been elucidated thus far and further studies will help us understand more about the acquired host response during epidermal restoration.

Regarding humoral responses and wound healing, it has been shown that B cells play a role in wound healing.[29] CD19 is suggested to regulate the contribution of B cells to wound healing by affecting TLR4 signaling, thereby altering cytokine production. Understanding the contributions of T and B cells to the wound repair process could provide new clues into regulation of wound re-epithelialization.

Bridge between innate and adaptive immunity

Both innate and adaptive immunity are required for cutaneous immunosurveillance and their role in wound healing is well established.[25] Increasing evidence suggests that induction of the adaptive immune response requires activation of innate immunity and a dependent relationship between the two systems.[30,31] Consequently, the bridge between the innate and the acquired immune systems has become an area of emerging exploration.

Although various elements have been shown to link the two immune responses, much of the overlap can be attributed to the activity of cells that demonstrate functional properties of both, including plasmacytoid dendritic cells (pDCs), gamma delta (+) T lymphocytes ($\gamma\delta$T), and Langerhans cells (LCs), key players in wound healing.

Plasmacytoid dendritic cells. Dendritic cells serve in innate immunity by utilizing PRRs and rapidly sensing the presence of PAMPs. They function as antigen-presenting cells, triggering the activation of naive T cells and thus the adaptive

immune response. pDCs are a specialized subset of human dendritic cells that produce large amounts of interferon type 1 (IFN-1), including α and β, in response to viral infections.[32] Their intracellular expression of TLR7 and TLR9, which recognizes single-stranded viral DNA and RNA of the invading virus, allows for IFN production to occur.[33] IFN-α/β acts as an innate effector by inhibiting viral replication and promoting entry into adaptive immunity by inducing maturation of dendritic cells. pDCs also have the ability to sense host-derived nucleic acids released in skin wounds.[34] They rapidly infiltrate both murine and human skin wounds and transiently produce type I IFNs via TLR7- and TLR9-dependent mechanisms suggesting that the aforementioned rapid infiltration and IFN production have a significant role in the induction of early inflammatory response and re-epithelization of wounded skin.[34]

Langerhans cells. LCs play a pivotal role in epidermal immune surveillance. Derived from the bone marrow, these antigen-presenting cells serve as first-line defenders and contribute to the induction of cutaneous immune responses. It has been proposed that LCs contribute to epidermal homeostasis and direct keratinocyte proliferation and subsequently promote its differentiation. Though LCs are reported to rapidly activate following the inflammatory response in wounding, little is known regarding the exact protective mechanisms of these cells. LC repopulation occurs early on in the process of re-epithelization in the murine and porcine model, and during epidermal injury, depleted LCs migrate from hair follicles.[35] In patients suffering from pressure ulcers, zinc supplementation leads to a more dendritic morphology of LCs suggesting that change in morphology may indicate a more motile state, thus affecting healing outcome.[36] In another study, the presence of CD1a + LC in normal skin and CWs was compared and accumulation at the border area of a DFU and a decrease in LCs at the edge of a VU was observed,[19] but their presence was not correlated to the healing outcomes. The identification of two populations of LCs earlier this year, one that populates the skin during steady state and the other, during inflammation,[37] necessitates further studies to elucidate the mechanisms behind LCs contribution to wound epithelialization.

$\gamma\delta$T cells. A subset of T cells, $\gamma\delta$T cells, expresses a T-cell antigen recognition receptor (TCR) made of γ and δ subunits, distinct from the better defined $\alpha\beta$T cells, and demonstrates features of both innate and acquired immunity. $\gamma\delta$T cells display considerable subset heterogeneity, with complex patterns of effector function that range from T-cell help to antigen presentation. The $\gamma\delta$T cell subpopulation within the epidermal compartment of the skin of mice is more commonly known as dendritic epidermal T cells (DETC). DETCs arise during fetal development and express an invariant and canonical Vγ3Vδ1 T-cell receptor. $\gamma\delta$T cells and DETCs have adaptive immune response properties in that they exhibit γ and δ gene rearrangement, which can ultimately result in a memory phenotype.[38] On the other hand, the use of restricted germline gene segments, functionally distinct cell populations in various tissues, antigen-presenting capabilities, and multiple interactions with other members of the innate immune system highlight the innate capabilities of these cells.[39] DETCs have a characteristic dendritic morphology enabling multiple contacts with neighboring keratinocytes and LCs. DETCs extend their dendrites to monitor keratinocytes for signs of damage or disease and retract them in response to keratinocyte damage, allowing for proliferation and migration of both DETCs and keratinocytes, crucial to the wound healing process (Fig. 3).[40–42]

Stress antigens represent likely candidate ligands for $\gamma\delta$T cells, whose TCRs have relatively limited diversity in large part because of the preferential pairing of particular V_γ and V_δ chains. In this way, $\gamma\delta$T cells can recognize subtle changes in quantity/quality of self molecules in conditions of stress, infection, epidermal injury, and others. Indeed, published work has shown that epidermal

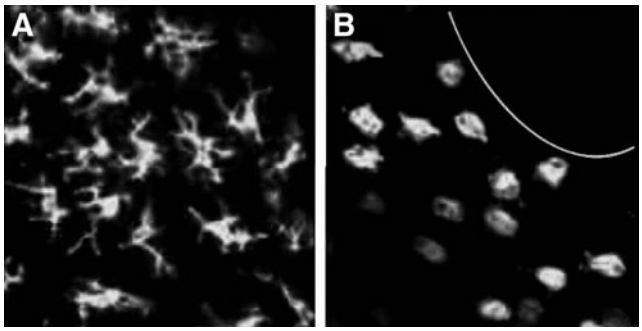

Figure 3. Dendritic epidermal T-cell (DETC) morphology changes in response to tissue damage. DETC present in normal epidermis **(A)** have a dendritic morphology. DETC located around a wound **(B)** become rounded and this morphology change correlates with initiation of a functional response. Epidermal sheets from a C57BL/6J mouse were stained with PE-anti-$\gamma\delta$ TCR (mAb GL-3) and confocal images were acquired with a 40× objective. Reproduced with permission from Havran and Jameson,[42] © 2012 The American Association of Immunologists, Inc.

$\gamma\delta$TCRs are continuously engaged and functionally activated in the steady state, with the TCRs simply undergoing relocalization after tissue damage.[40] Such a mechanism allows a rapid response to stress cues. However, only a few stress-induced molecules have actually been identified as ligands for $\gamma\delta$T cells (Rae-1, Plexin B2, and CAR; Fig. 4).[43–49]

Signals delivered between keratinocytes and DETCs are crucial for effective wound healing. TCR-specific activation is pivotal to DETC function during cutaneous wound repair.[50] Published studies have identified NKG2D,[51] JAML,[52] and CD100 (Sema 4D)[47] as co-stimulatory molecules for DETC, known to interact with damaged keratinocytes in response to wounding (Fig. 4). Mice deficient in epidermal Vγ3Vδ1 DETCs have been shown to lack adequate barrier protection[53] and demonstrated delayed wound closure and re-epithelialization.[41] Furthermore, compared with normal T cell production of insulin growth factor-1 (IGF-1), a key regulator of early acute wound healing, $\alpha\beta$ and Vδ1T cells isolated from CWs produced no IGF-1 even upon stimulation, thus suggesting their unresponsive state.[54]

Upon keratinocyte damage, an as yet uncharacterized antigen is expressed on the surface of the keratinocytes and recognized by the canonical TCR expressed by DETCs.[55] Using soluble DETC TCR tetramers, Komori *et al.* demonstrated that DETC TCR ligands are not constitutively expressed in healthy tissue, but are rapidly upregulated on keratinocytes bordering wound edges following wounding. Early inhibition of TCR-ligand interactions using DETC TCR tetramers delays wound repair *in vivo*, highlighting DETCs as rapid responders to epidermal injury.[55]

Recent identification of the costimulatory pair junctional adhesion molecule-like protein (JAML) and Coxsackie and Adenovirus receptor (CAR)[52,56] provided direct evidence that, similar to $\alpha\beta$T cells, complete activation of DETCs requires coordinated interaction of co-stimulatory molecules in addition to the TCR-specific activation. JAML is upregulated on activated DETCs and CAR expression is upregulated on keratinocytes located around wounds in normal mice for several days. Inhibition of JAML–CAR-mediated co-stimulation leads to diminished $\gamma\delta$T cell activation and delayed wound closure similar to that seen in the absence of $\gamma\delta$T cells.[52] Furthermore, plexin B2 was found to exert its effect on DETCs through interaction with the semaphorin ligand CD100.[47] CD100 mediates $\gamma\delta$T cell morphology changes both *in vitro* and *in vivo* and plays a fundamental role in wound repair. Mice deficient in CD100 showed a defective $\gamma\delta$T cell response to keratinocyte damage resulting in delayed cutaneous wound healing.[47]

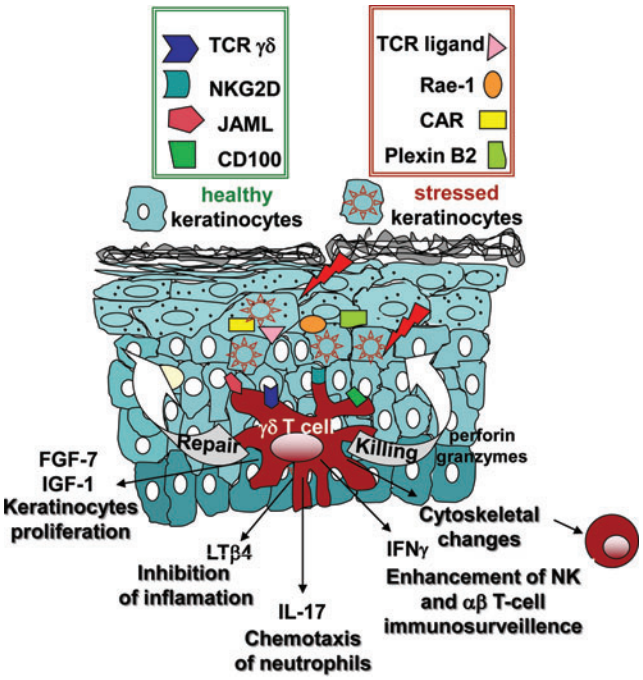

Figure 4. $\gamma\delta$ T cells in murine skin. $\gamma\delta$ T cells recognize damaged keratinocytes as those cells displaying the TCR ligand and ligands for NKG2D (*e.g.*, retinoic acid early-1 (Rae-1), ligands for JAML (CAR), and ligands for CD100 (plexin B2). Such cells can be rapidly eliminated because skin $\gamma\delta$T cells expressing perforin and granzyme, exert cytolytic functions against virus- or parasite-infected, transformed or stressed keratinocytes.[43,44] Concurrently, $\gamma\delta$ T cells may promote wound healing through the release of FGF7, KGF-1, and IGF-1[45] stimulating neighboring healthy keratinocytes to proliferate. Further, the local secretion of IFN-γ by $\gamma\delta$T cells[46] enhances the antimicrobial, antitumor, and other functional activities of NK and $\alpha\beta$ T cells, thus maintaining epidermal integrity. CD100 mediates $\gamma\delta$T cell morphology changes both *in vitro* and *in vivo* and plays a fundamental role in wound repair.[47] Motile dermal $\gamma\delta$T cells constitutively expressed the IL-23 receptor (IL-23R) and transcriptional factor RORγt and produce IL-17[48,49] and may be critically involved in cutaneous inflammatory responses. CAR, coxsackie and adenovirus receptor; JAML, junctional adhesion molecule-like protein; IGF, insulin growth factor; FGF, fibroblast growth factor; KGF, keratinocyte growth factor. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Since DETCs are ideally localized to monitor the epidermal damage, it seemed likely that they could contribute to tissue repair.[57] Activated DETCs inducibly secrete keratinocyte growth factors (KGF-1/FGF-7 and KGF-2/FGF-10), potent epithelial cell mitogens. KGF-1 acts specifically on epithelial cells, including keratinocytes, to stimulate proliferation and migration of these cells during wound healing.[57] In addition, activated DETCs produce chemokines that may affect migration of cells into the epidermis following trauma. These data support DETCs participation not only in maintenance of epithelial homeostasis but also in tissue repair (Fig. 4).

DETCs appear to be a rich source for a variety of cytokines and chemokines, which are mainly proinflammatory. Recently, it was reported that motile dermal $\gamma\delta$T cells constitutively express the interleukin-23 receptor (IL-23R) and transcriptional factor RORγt.[48] Further analyses will be helpful to gain more insight into the regulation, limitation, and plasticity of DETC signature cytokines. The ability to produce many inflammatory cytokines suggests critical involvement of DETC in cutaneous inflammatory process and consequently, wound epithelialization.

Inflammasomes

Inflammasomes are multi-protein complexes that, upon recognizing a diverse set of stimuli, result in the production of proinflammatory cytokines, including IL-1β and IL-18.[58] Inflammasomes have been shown to regulate inflammation and epithelial cell regeneration in the mucosa, particularly in the gut.[59] Similarly, the investigation into the role of inflammasomes in wound re-epithelization has begun, but much remains to be explored. A role for Absent in melanoma 2 (AIM2), a cytosolic sensor of double-stranded DNA, has been suggested in the inflammatory environment and pronounced induction of epidermal AIM2 in subpopulations of epidermal chronic wound keratinocytes was reported. Considering our limited knowledge regarding epidermal inflammasomes and yet their documented presence in epidermal keratinocytes, one can speculate that this molecular complex plays a role in wound closure.

TAKE-HOME MESSAGES

- Successful re-epithelialization in the wound healing process requires a well-balanced immune response and its aberrant expression and/or activity leads to delayed wound closure.
- TLRs, mediators of the innate immune response, differentially regulate the wound healing process. TLR2 activation results in delayed wound closure, while induction of TLR9 accelerates re-epithelialization. In this regard, the immunologic contribution of keratinocytes to wound closure includes recognition of microorganisms by toll-like receptors and the subsequent production of inflammatory mediators.
- A role for T and B cells in stimulating wound healing has been established.
- The elements bridging the innate and adaptive immune systems have recently emerged as critical regulators of re-epithelialization.
- Plasmacytoid dendritic cells induce an early inflammatory response to stimulate optimal wound repair and re-epithelialization.
- LCs repopulate the wound epidermis during early wound healing to promote re-epithelialization.
- DETC exert their pleiotropic effects and assist re-epithelialization by secreting growth factors and cytokines. Animals deficient in DETCs or DETC ligands demonstrate delayed wound closure.
- Identification of the critical roles of immune elements in wound re-epithelialization will provide novel insights to facilitate the development of therapeutic targets and improve clinical outcomes.

CONCLUSION

The innate and adaptive immune systems have been explored in terms of their contributions to wound healing. A fully functioning immune system is essential to create an optimal wound healing environment by generating an appropriate inflammatory response and successful wound closure. Equally critical to wound healing and skin homeostasis appear to be the elements bridging the two. The role of the immune system pertaining to wound re-epithelialization specifically, however, deserves further investigation. The data supporting adaptive immunity, in particular, in regenerating wound epithelia are sparse.

Furthermore, much of the data presented are extrapolated from *in vitro* and *in vivo* models. Further human studies are necessitated to elucidate the interplays between the receptors, immune and epithelial cells during the healing process.

Ultimately, much has been recently uncovered regarding the vital role of immune cells and their interaction with surrounding cells, for example, keratinocytes in the restoration of wounded epidermis. The potential use of these molecules as biomarkers of chronic, nonhealing wounds may provide increased insight into the prognosis of disease severity, progression, and ultimately treatment options. Immunostimulatory or suppressing molecules, depending on the individual's wound milieu, may also provide novel therapeutic strategies for accelerating wound healing. There is a delicate immunologic balance in skin epidermis, which deserves further exploration to develop new therapeutic regimens and improve clinical outcomes.

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AUTHOR DISCLOSURE AND GHOSTWRITING

No competing financial interests exist. The content of this article was expressly written by the authors listed. No ghostwriters were used to write this article.

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Natasa Strbo, MD, PhD, is an Assistant Scientist at the Department of Microbiology and Immunology, University of Miami. Dr. Strbo is a well-trained immunologist with excellent track record in immunology with over 28 original research articles published in peer reviewed immunological journals. Dr. Strbo research interests are focused on mucosal immunity and HIV vaccine development. She has developed and established a cell-based secreted heat shock protein gp96 based SIV/HIV vaccine approach in mice and nonhuman primates. Her recent translational research interests aim to understand cellular and molecular basis of $\gamma\delta$T cells in different mucosal tissues, including skin, gut, and reproductive tract. **Natalie Yin, BS,** is a current medical student and research fellow in the Department of Dermatology and Cutaneous Surgery at the University of Miami. **Olivera Stojadinovic, MD,** currently holds a faculty appointment as a Research Assistant Professor at the Department of Dermatology and Cutaneous Surgery at the University of Miami. Dr. Stojadinovic's research interests are focused on molecular mechanisms that control inflammatory response in skin biology and its diseases, and understanding how control of cortisol metabolism may modulate skin inflammation and response to injury. Her translational research interests aim to understand cellular and molecular basis of wound healing and its immunopathogenic mechanisms and how it may correlate with clinical outcomes.

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