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Wendy L. Havran and Julie M. Jameson

The murine epidermis contains resident T cells that express a canonical γδ TCR. These cells arise from fetal thymic precursors and use a TCR that is restricted to the skin in adult animals. These cells assume a dendritic morphology in normal skin and constitutively produce low levels of cytokines that contribute to epidermal homeostasis. When skin is wounded, an unknown Ag is expressed on damaged keratinocytes. Neighboring γδ T cells then round up and contribute to wound healing by local production of epithelial growth factors and inflammatory cytokines. In the absence of skin γδ T cells, wound healing is impaired. Similarly, epidermal T cells from patients with healing wounds are activated and secreting growth factors. Patients with nonhealing wounds have a defective epidermal T cell response. Information gained on the role of epidermal-resident T cells in the mouse may provide information for development of new therapeutic approaches to wound healing. The Journal of Immunology, 2010, 184: 5423–5428.

Epithelial tissues line the external and internal surfaces of the body and provide an effective environment to protect the organism from the outside world. These tissues not only provide barrier functions, but also contain resident populations of cells with unique functions that contribute to homeostasis, surveillance, protection, and repair of the epithelia. Epithelial tissues, including the skin, intestine, and lung are the largest organs in the body and, together, are the residence of the vast majority of lymphocytes in the body (1). Some of these immune cells have specialized functions related to their epithelial residence, including the IgA-producing B cells of the intestine and the γδ T cells. There is a resident population of γδ T cells in the epithelial tissues of all mammalian species (2). In contrast to the blood and peripheral lymphoid tissues where γδ T cells are typically a minor population, γδ T cells are the only resident lymphocytes in the murine epidermis. In other epithelial tissues, including the intestine and lung, the γδ T cells coexist with αβ T cells and other lymphocyte populations. Recent evidence from numerous laboratories has shown specialized roles for these γδ T cells in maintenance of epithelial homeostasis and response to tissue damage, infection, inflammation, and malignancy (3–5).

The epidermis is the outermost layer of skin. Murine epidermis is home to a unique population of γδ T cells, dendritic epidermal T cells (DETCs). DETCs express a canonical Vγ3Vδ1 TCR (alternate nomenclature Vγ5Vδ1) that is only expressed on these skin-resident T cells. This lack of TCR diversity and skin-specific localization suggests a potential limited repertoire of skin-expressed Ags for the DETC that may direct DETC functions in the epidermis (4, 6).

The epidermis is under constant exposure to UV light, chemicals, allergens, and traumatic injury. Effective tissue repair requires cooperation of multiple cell types to produce varied growth factors and perform effector functions that orchestrate healing. Recent results have shown critical roles for DETCs in recognition and response to epidermal injury (4, 6). An increasing number of patients have chronic, nonhealing wounds. The causes are not well understood, and treatment strategies are often not satisfactory. Obtaining a better understanding of the contributions of DETCs and other immune cells to wound healing may lead to development of effective new strategies for treatment of chronic wounds.

Development and homeostasis of epidermal γδ T cells

There are several key features of the development and homeostasis of DETCs that contribute to their roles in wound healing. Strikingly, the TCR γ and δ genes are rearranged and expressed in an ordered manner during thymic ontogeny, and T cells expressing specific Vγ and Vδ gene pairs migrate from the developing thymus to take up residence in specific epithelial tissues (Fig. 1). A series of programmed differentiation events, coupled with cellular selection processes, proceed in a systematic order to produce functional T cells (reviewed in Refs. 7 and 8). The γδ T cells that localize in epithelial tissues have mainly tissue-specific TCRs with limited or no diversity. This is in sharp contrast with the highly diverse γδ TCRs expressed by γδ T cells found in peripheral lymphoid organs and blood. The first TCR genes that are expressed on developing murine fetal thymocytes are Vγ3 paired with Vδ1. Unexpectedly, the TCR expressed by these cells is invariant with no junctional diversity because of the lack of expression...
of terminal deoxynucleotidyl transferase, gene accessibility, and recombination signal sequence restrictions at this stage of fetal development, coupled with cellular selection processes (7, 9, 10). This results in a limited window of time in which these TCR genes are accessible for rearrangement, effectively limiting development of Vγ3Vδ1+ thymocytes to a discrete stage of development. Vγ3Vδ1+ thymocytes are not generated in the adult thymus. These Vγ3Vδ1+ cells migrate from the fetal thymus to the epidermis where they expand to homeostatic numbers (7), conform to a dendritic morphology to facilitate interactions with multiple neighboring epithelial cells, and remain throughout life.

The epidermis is the only site in the adult mouse containing cells that express this TCR. Because DETCs are the only resident T cells and the Vγ3Vδ1 TCR is invariant, the epidermis is populated by a monoclonal population of T cells. This complete absence of receptor diversity suggests that only a single or limited repertoire of Ags can be recognized by DETCs. Recognition of a single foreign peptide Ag would severely limit the potential functional effectiveness of this population of cells, suggesting the possibility of recognition of ligands quite different from those seen by αβ T cells. Ags for γδ T cells, including DETCs, are not well characterized and no paradigm exists to provide clues about Ag candidates. However, evidence from multiple laboratories indicates that DETCs recognize self-ligands expressed by neighboring keratinocytes after damage or disease (3, 4, 6). Recognition of a conserved consequence of trauma may allow these monoclonal T cells to be broadly reactive to any form of epithelial stress rather than highly specific for a single pathogen. Studies are in progress to identify the specific keratinocyte Ag recognized by DETCs.

In addition, cytokines play key roles in DETC development by providing differentiation, proliferation, survival, and migration signals. Mice deficient in expression of IL-2Rβ, IL-7, IL-7R, IL-15, and IL-15R all have reduced numbers or lack DETCs in the skin (11, 12). Also, mice that lack expression of transcription factors Runx3, which regulates expression of IL-2Rβ, and the E protein E47, which regulates IL-7R expression, have defects in γδ T cell development and lack DETCs (13, 14). IL-2 and IL-15 provide essential signals for survival and expansion of DETC precursors in the fetal thymus and after migration to the skin (11, 12). DETC precursors express IL-7R, and signals provided by IL-7 have been shown to promote recombination and transcription of TCR γ genes as well as increased expression of antiapoptotic proteins (12). These cytokine signals coordinate with molecular rearrangement mechanisms to allow DETC development to proceed.

There has been some controversy over the years about the role of cellular selection in DETC development. Development of T cells in the epidermis is not dependent on expression of the Vγ3 or Vδ1 TCR because mice deficient in these genes had DETCs with alternate TCR and mice lacking all γδ T cells have αβ TCR-expressing DETCs (15). There is evidence that positive selection is necessary to coordinate expression of chemokine receptors, such as CCR10/CCL27, and cytokine receptors, including IL-7R and IL-15R, that control thymic egress and homing to the epidermis (16). The Skint gene family was recently described to be expressed only by epithelial cells in the skin and thymus (17). Mice with a spontaneous mutation in Skint1 have greatly reduced Vγ3Vδ1+ DETCs in the epidermis. Skint1 does not appear to directly bind to the TCR but is able to mediate critical interactions during DETC development. Although the mechanism of action is not fully defined, the Skint1 gene product is clearly required for maturation and expansion of DETC precursors in the thymus. The restrictions in early fetal gene rearrangement with specific signals from cytokines and Skint1 work together to provide the early window during ontogeny for development of DETCs that are missing in the adult.

Roles of epidermal T cells during tissue repair
γδ T cells have been implicated as early and rapid responders to tissue damage. Their location in barrier tissues, such as the skin, lung, and intestine, makes them an ideal candidate for participating in protecting the organism from infection and maintaining tissue homeostasis. Efficient restoration of barrier function is precisely orchestrated with a complexity that has been frustratingly difficult to replicate in patients with nonhealing wounds. The earliest events in wound repair responses include cellular damage itself, which initiates stress signals relayed by the epithelial cells residing at the front line of injury. Damaged, stressed, or transformed keratinocytes express an unknown Ag that activates DETCs in a TCR-mediated fashion in cell culture. A rapid response by skin γδ T cells is evident in vivo by the dynamic morphology change of the epidermal γδ T cells within hours of tissue damage (18) (Fig. 2 and unpublished data). These early activation responses in wound repair by the epithelial cells themselves and their adjacent γδ T cell neighbors cue responses by other cells types in the wound environment.

Several models of wound healing have been established to investigate the role of DETCs in wound repair in vivo, including full-thickness punch biopsy wounds and burn wounds. Mice lacking γδ T cells exhibit a delay in wound closure when administered punch biopsy wounds (18). This defect is attributed to the lack of growth factors, such as keratinocyte growth factors (KGFs), which are important for keratinocyte proliferation. Fewer keratinocytes rapidly proliferate at the wound site in γδ T cell deficient mice as compared with control mice. γδ T cells isolated from the site of tissue damage express...
KGF-1 and KGF-2 RNA (18), produce TNF-α, and upregulate activation markers, such as CD25 (19). The response of γδ T cells to damaged keratinocytes is rapid, as rounding of the DETCs can be detected within 4 h and cytokine production within 24 h with KGF production following after 48 h. This timing of activation correlates well with the defect in wound repair in TCRδ−/− mice, which is most evident in the earliest stages of wound repair, resulting in a 2–3 d delay in complete wound closure (18). Epidermal T cell responses to tissue damage in mice require the keratinocyte-responsive γδ TCR, as αβ TCR+ cells that reside in the epidermis of TCRδ−/− mice are unable to produce cytokines at the wound site (19).

Wound repair occurs in a series of four phases that are required for rapid and complete healing. Healing is initiated by the development of a fibrin clot. This is followed by the inflammatory phase in which various cell types, such as neutrophils, macrophages, and T cells, infiltrate the wound site over a 7-d period. The epithelialization phase proceeds in response to factors produced by both resident and infiltrating cells. The final phase of tissue repair is the remodeling phase that can extend for weeks or longer as extracellular matrix is deposited and a scar is formed. Regulation of epithelialization or keratinocyte proliferation and migration during wound repair is important for modulating skin closure. Growth factors, such as KGF-1 and KGF-2, are known to regulate keratinocyte proliferation, but are not produced by keratinocytes themselves. Skin γδ T cells produce both KGFs and insulin-like growth factor (IGF)-1 within 2 d postinjury, which positively impacts the number of proliferating keratinocytes (20). DETC participation in wound repair is regulated by the serine threonine kinase mammalian target of rapamycin (21). Mice treated with rapamycin, an inhibitor of mammalian target of rapamycin signaling, exhibit a delay in wound healing and defects in DETC rounding and cytokine production at the wound site.

γδ T cells exhibit immunoregulatory functions when skin homeostasis is disrupted by damage or inflammation. DETCs regulate the infiltration of αβ T cells into the skin during atopic dermatitis, contact hypersensitivity reactions, and following wounding (6, 22). Migration of inflammatory cells into the wound site is required for complete and efficient wound repair. Mice lacking γδ T cells exhibit delayed infiltration of macrophages into the wound site (22). The decreased expression of KGFs because of the absence of γδ T cells results in decreased deposition of extracellular matrix molecules, such as hyaluronic acid (23). Addition of KGF to the wound site restores hyaluronan levels. Hyaluronan is produced by both keratinocytes and skin γδ T cells. Conversely, molecules produced by γδ T cells have also been implicated in anti-inflammatory responses (23). Skin γδ T cells were shown to produce a lymphoid-associated thymosin-β4 variant that was able to suppress inflammation associated with contact dermatitis.

γδ T cells also participate in several aspects of healing from burn injuries (24). Mice lacking γδ T cells have a defect in the inflammatory phase of wound healing. Entry of inflammatory populations, such as macrophages, into the burn site is delayed in burn wounds from TCRδ−/− mice. Recently this defect in macrophage entry into the burn site has been attributed to the DETC (25). Peripheral γδ T cells also play a role in neutrophil infiltration of other tissues during the inflammatory phase of wound repair (22). Together, the full-thickness wound and burn injury models suggest that DETCs participate in tissue repair, likely through the production of growth factors, cytokines, and chemokines that regulate the epithelia and modulate migration of inflammatory cells.

The T cell composition of the epidermis differs between humans and most other mammals. Although most mammals exhibit primarily γδ T cells in the epidermis, both αβ and γδ T cells are resident in human skin (26–31). The dermal T cells have been best characterized in man, but T cells are also found in the epidermis at a ratio of ~5 αβ to 1 γδ T cell (31). Reports published from as early as 1920 claim that 2–40% of human epidermal cells, at a variety of physical locations, are T cells (29, 30). These conflicting reports over the years regarding the presence of T cells in human epidermis may be due in part to technical difficulties as many of the mAb used for the detection of T cells do not work well in immunohistochemical techniques. This has led to speculation that humans do not have a population of T cells in the epidermis with wound repair capabilities. However, this has recently been challenged as both αβ and γδ T cell populations isolated from human epidermis produce growth factors, such as IGF-1, constitutively, and epidermal T cell production of growth factors is increased after in vitro stimulation. There is active secretion of growth factors in both T cell populations isolated from the skin of patients with acute wounds suggesting that the epidermal T cells may contribute to wound repair (31). Indeed, T cell stimulation increases the efficiency of tissue repair in wounded human skin cultured in vitro. In contrast, T cells isolated from chronic wounds do not produce IGF-1 and are not responsive to stimulation. These cells are unable to produce IL-2 and other cytokines on ex vivo stimulation suggesting that the normal TCR signaling pathway is impaired in patients with nonhealing wounds.

Although both αβ and γδ T cells isolated from human epidermis can produce cytokines and growth factors, it will be important to determine whether human epidermal αβ and γδ T cells exhibit differential functions. It is interesting to speculate that the primary role of the αβ T cell population may be to provide a defense against pathogens, whereas the γδ T cells may primarily function to regulate keratinocyte growth and survival. Understanding how these skin-resident T cell populations function may lead to identification of novel targets for therapeutic intervention in patients who have chronic, nonhealing wounds.
Molecules regulating epidermal T cell functions

Ags for skin γδ T cells remain unknown. At this time little is known about Ags for any epithelial-resident γδ T cell population and no paradigm exists to provide clues to this critical information. The Skin1 gene product was an attractive candidate molecule for a DETC Ag because of its role in DETC development, but recent data does not support DETC TCR binding to this molecule (3). However, several lines of evidence do support the hypothesis that DETC Ags are expressed on keratinocytes located in wounded tissues. DETCs in areas proximal to a wound site lose dendritic morphology and become rounded several hours post wounding, whereas those T cells distal to the wound remain dendritic (Fig. 2) (18). A comparison of DETCs found around wounds with those in nonwounded areas indicates that morphology correlates with functional activity. The rounded DETCs found near wounds are activated and secrete a variety of cytokines and growth factors, whereas those distal to wounds retain a resting phenotype and secrete homeostatic levels of factors (18). Preliminary data using a soluble form of the DETC TCR ligand indicates that DETC Ags are expressed rapidly and transiently on local keratinocytes following wounding (H.K. Komori, D. A. Witherden, J.M. Jameson, L. Teyton, and W.L. Havran, submitted for publication). These data fit nicely with published studies showing that DETCs, and other epithelial resident γδ T cells, are “slightly activated” under normal conditions, express CD69 and CD25, as well as secrete low levels of certain cytokines (32), in contrast to typical T cell populations. This partial activation allows the DETC to be poised for fast, full activation in response to local trauma.

DETCs do not express CD4, CD8, or CD28. The absence of typical sources of coreceptor and costimulatory signals for T cells raises the possibility that other molecules may contribute to full DETC activation. The activating receptor NKG2D is expressed by all γδ T cells, including DETCs. In general, NKG2D ligands are not significantly expressed by normal cells but can be upregulated after damage or disease raising the possibility for a role for NKG2D-mediated signals in DETC responses to wounding (3). A large number of ligands have been described for NKG2D and somewhat controversial data exists as to whether signals delivered by recognition of these ligands provide direct stimulation or costimulation to T cells. A new NKG2D ligand, H60c, was recently shown to be specifically expressed in the epidermis and on cultured keratinocytes (33). H60c was shown to provide potent costimulatory signals to DETCs in vitro but was not sufficient to activate in the absence of TCR-mediated signals (33). Future work should determine whether this ligand is upregulated on wounded keratinocytes and any role NKG2D-mediated costimulation may play in DETC wound healing functions.

We have recently identified a new costimulatory molecule for DETCs that shares a signaling motif with CD28 (D.A. Witherden, P. Verdino, S.E. Rieder, O. Garijo, R.E. Mills, L. Teyton, W.H. Fischer, I.A. Wilson, and W.L. Havran, manuscript in revision; and P. Verdino, D.A. Witherden, W.L. Havran, and I.A. Wilson, manuscript in revision). Functional adhesion molecule-like protein (JAML) is a member of the junctional adhesion molecule family and is expressed by DETCs. DETC recognition of the JAML ligand coxsackie and adenovirus receptor expressed by keratinocytes results in the in vitro and in vivo costimulatory activation. This JAML-mediated costimulation was shown to provide signals necessary for effective DETC participation in wound healing (D.A. Witherden, et al., manuscript in revision).

These results suggest that rules for DETC activation differ significantly from typical αβ T cell activation requirements. The poised and ready nature of these cells fits well with recognition of unique Ags that are rapidly and transiently expressed. Evidence suggests that the unknown Ag is not a peptide that requires processing and presentation by MHC molecules (4, 34, 35), which does not occur rapidly. Recognition of costimulatory ligands whose expression is regulated in response to damage may provide an additional level of specialization for these cells. Together, the recognition of damage-induced Ags and costimulatory ligands appears to regulate effector functions of DETCs that contribute to tissue repair in the epidermis (Fig. 3).

Roles of other γδ T cells in tissue repair

Tissue-resident γδ T cells have been implicated in the repair of epithelia in other organs, such as the intestine, lung, and cornea. Interestingly a population of γδ T cells has also been implicated in repair of skeletal injury (36). Various models have been established in these tissues to induce epithelial damage. The chemical dextran sulfate sodium salt (DSS) has been used to inflict damage on the intestinal epithelia, so that healing can be monitored once the chemical is removed from the drinking water (37). In the absence of γδ T cells, DSS-treated mice exhibit more severe disease and defects in the repair of intestinal damage (38, 39). Similar to the skin, epithelial cell proliferation in the intestine is known to restore barrier integrity. However, mice lacking γδ T cells exhibit decreased epithelial cell proliferation in response to DSS damage (38). A second method of inducing colitis is the intrarectal injection of 2,4,6-trinitrobenzene sulfonic acid. Depletion of γδ T cells in mice treated with 2,4,6-trinitrobenzene sulfonic acid results in increased mortality and more severe illness (40).

FIGURE 3. Model of DETC functions during skin homeostasis and wound healing. DETCs secrete low levels of cytokines in normal skin that contribute to epithelial homeostasis. Unknown Ags for DETCs are expressed on keratinocytes in wounded tissue. DETC recognition of Ag and costimulation through molecules like JAML-binding coxsackie and adenovirus receptor leads to DETC rounding and activation to produce cytokines, growth factors and chemokines that contribute to a wound-healing response.
To identify whether KGFs also play a role in repair of the intestine, KGF−/− mice were examined in the DSS-induced colitis model. KGF−/− mice exhibit more severe illness and decreased levels of epithelial cell proliferation posttreatment (38). Thus, KGF is an important growth factor in the intestine as well as the skin. Therapeutic intervention with KGFs was shown to greatly reduce mortality and improve weight loss in DSS-treated mice and rats (41–43) and a recently developed bacterial delivery system may overcome some of the problems with stability that have plagued the use of growth factors to improve colitis in patients (44).

γδ T cells also reside within the epithelia of the lung (45), where they have been shown to play roles in controlling infection and asthma (46–48). Mice lacking γδ T cells are more susceptible to infection by Nocardia asteroides, suggesting a role for γδ T cells in limiting infection (49). Data indicate that lung-resident γδ T cells may control infection by cytolsis and IFN-γ production (50, 51). Although the mechanisms of defense against pathogens may initially be direct and proinflammatory, there is also evidence that γδ T cells downmodulate inflammatory responses to infectious agents thus preventing further damage to surrounding tissue. These anti-inflammatory functions include regulation of macrophage infiltration (52) and production of IL-10 (53).

This dual role for γδ T cells also appears to be the case in the enhancement and suppression of airway hyperresponsiveness (AHR). One subset of γδ T cells, Vγ1+ γδ T cells, can enhance AHR (54), whereas another subset, Vγ4+, is able to suppress AHR on induction (46). The development of γδ T cells that modulate AHR is dependent on CD8α+ dendritic cells (55). In addition to pro- and anti-inflammatory roles, γδ T cells in the lung have been implicated in the repair of tissue after damage incurred by infection or ozone treatment (49). This suggests that similar to the skin and intestine, γδ T cells in the lung exhibit healing functions. Additional models of lung injury further support a healing role for γδ T cells in the lung. Bleomycin treatment induces epithelial damage and is followed by pneumonitis and fibrosis. TCRδ−/− mice exhibit defects in both inflammation and epithelial repair in the lung after bleomycin treatment (56). In addition, exposure to chlorine gas induces an increase in epithelial shedding in mice lacking γδ T cells (57). Taken together, these studies indicate that lung γδ T cells play key roles in modulating the responses of the lung epithelia to various insults.

An additional model of epithelial repair has identified a role for γδ T cells in the epithelia of the cornea. Corneal repair is vital to the healing of abrasions on the surface of the cornea. γδ T cells make up a large portion of the resident limbal epithelial T cells. Similar to other epithelial tissues, mice lacking γδ T cells exhibit defects in the migratory and proliferative phases of corneal epithelial repair (58). In addition, TCRδ−/− mice exhibit reduced platelet localization to the limbus and reduced inflammation. ICAM-1 is expressed by corneal epithelium and functions as an adhesive ligand for LFA-1—dependent migration or retention of γδ T cells into damaged tissue (59). The role of γδ T cells in barrier tissue maintenance and regeneration is becoming clearer; however, gaining knowledge about the molecules on γδ T cells that activate these responses and the disease environments that impair these responses require further investigation.

Conclusions
DETCs are an intriguing population of T cells with important roles in tissue repair. Significant key information is still unknown about TCR ligand(s) and the role of Ag recognition in development and function of these cells. It is hoped that as new information is obtained, this will contribute to the formation of a new paradigm for γδ T cell activation. Determination of γδ T cell contributions to wound healing in the mouse raised the possibility of similar functions in humans. Recent results demonstrating similar wound healing contributions by human epidermal T cells, and defective T cell function in patients with chronic, nonhealing wounds, have identified new potential targets for therapeutic intervention. Chronic wounds are an increasing clinical problem, and information gained about the role of DETCs in tissue repair in the mouse should help define new clinical treatment strategies.

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