of the spätzle ligand for the *Drosophila* Toll receptor that is generated via a serine protease cascade (*24*). In mature flies, Toll also functions in immunity to fungal pathogens, and derepression of this pathway, due to the absence of the Spn43Ac protease inhibitor, also results in necrosis (*25*).

Generating a mature ligand is unlikely to be the sole function of Rcr3, because *rcr3* mutants do not exhibit an HR when infiltrated with intercellular fluid preparations that contain Avr2 isolated from infected tomato plants (*2*).

Alternatively, Rcr3 could process Cf-2 or another plant protein. Differences in the substrate specificity or activity of $Rcr3^{pim}$ and $Rcr3^{esc}$ might explain why $Rcr3^{esc}$ induces Avr2-independent necrosis. An extracellular protease is also required for brassinosteroid perception in *Arabidopsis* (*26*). Overexpression of the serine carboxypeptidase BRS1 suppresses extracellular domain mutants of the BRI1 LRR-receptor kinase. BRS1 may process a protein that forms part of the BRI1 ligand (*26*). Alternatively, Rcr3 might be a plant defense component that is inhibited by Avr2. Avr2 could also inhibit other cysteine proteases, either by binding to or by modifying them. Whether Rcr3 has a role in defense is not established. The Cf2 Rcr3 mutant lines do not appear more susceptible to *C. fulvum* than Cf0, but tomato encodes many different cysteine proteases. It is interesting that the *C. fulvum* Avr9 protein shows significant structural homology to several protease inhibitors (*27*).

It is possible that Avr2 and Rcr3 together constitute a complex ligand that is recognized by Cf-2. It has been suggested that R proteins act as 'guards' for specific proteins targeted by pathogen Avr proteins during infection (*1*, *2*). Cf-2 may guard Rcr3 and trigger a defense response upon perception of an Rcr3/Avr2 complex. In *rcr3* mutants, no Avr2-independent signaling would occur either because no $Rcr3^{pim}$/Cf-2 complex is formed or because the complex does not activate defense signaling. A subtle structural difference in $Rcr3^{esc}$ (Fig. 1A) may result in activation of an Avr2-independent response upon binding to Cf-2.

With the recent isolation of the Avr2 gene (*28*), it will be possible to determine whether the Avr2, Rcr3, and Cf-2 proteins can interact. This should further increase our understanding of the molecular mechanism of ligand perception by this unique class of R proteins.

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7. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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A Role for Skin γδ T Cells in Wound Repair

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γδ T cell receptor–bearing dendritic epidermal T cells (DETCs) found in murine skin recognize antigen expressed by damaged or stressed keratinocytes. Activated DETCs produce keratinocyte growth factors (KGFs) and chemokines, raising the possibility that DETCs play a role in tissue repair. We performed wound healing studies and found defects in keratinocyte proliferation and tissue reepithelialization in the absence of wild-type DETCs. In vitro skin organ culture studies demonstrated that adding DETCs or recombinant KGF restored normal wound healing in γδ DETC-deficient skin. We propose that DETCs recognize antigen expressed by injured keratinocytes and produce factors that directly affect wound repair.

γδ T cells compose a major T cell component in epithelial tissues (*1*, *2*). The tight correlation between T cell receptor (TCR) V gene segment usage and tissue localization suggests a highly specialized function. γδ TCR-bearing DETCs found in murine skin produce cytokines and proliferate in response to damaged or stressed keratinocytes (*3*), indicating a functional interaction between these two neighboring cell types in vivo. DETCs produce KGF-1, also called fibroblast growth factor-7 (FGF-7), following stimulation through the γδ TCR (*4*). Both FGF-7 and KGF-2 (FGF-10) bind the FGFR2-IIIb receptor and have been implicated in wound healing (*5*–*15*). To directly test the potential role of γδ T cells in wound repair, we set up wound healing studies in mice lacking γδ DETCs.

Location, morphology, and density of DETCs were evaluated after wounding of C57BL/6 mouse ear skin (*16*) (Fig. 1, A through C). Twenty-four to 48 hours after full-thickness wounding, DETCs located around the wound exhibited a change in morphology characterized

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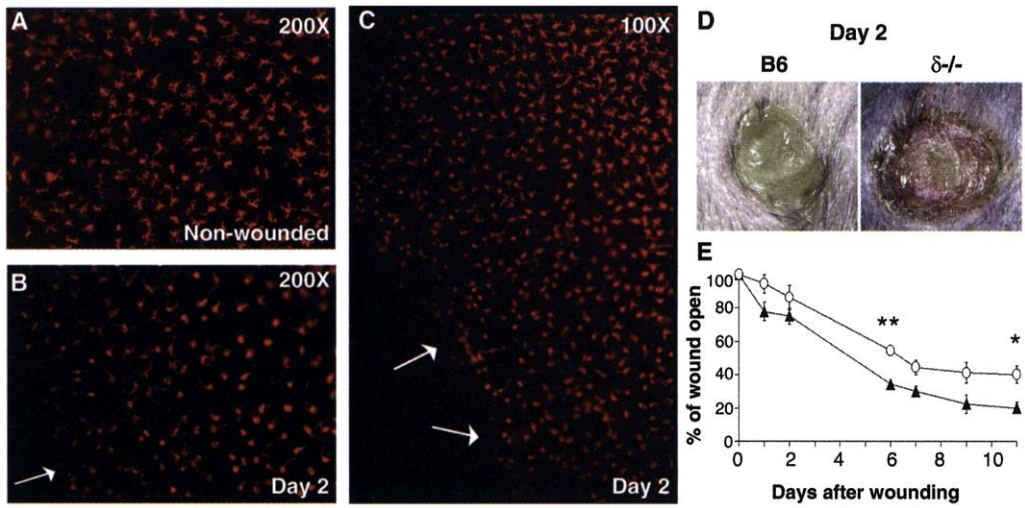

Fig. 1. γδ DETCs participate in wound repair. (A through C) Detection of DETCs in epidermal sheets isolated from C57BL/6 (B6) mice with a γδ TCR-specific mAb (PE-GL3; Pharmingen, La Jolla, CA) (*16, 27, 28*). Arrows indicate the wound edge. (**A**) Nonwounded (magnification ×200). Day 2 after wounding (**B**) ×200 and (**C**) ×100. Data are representative of wounds examined from at least 10 mice. (**D**) Photographic images of excision wounds from B6 and TCRδ$^{-/-}$ mice 2 days after wounding. (**E**) Wound closure kinetics in wild-type (▲) and TCRδ$^{-/-}$ mice (○). Data are representative of at least three experiments. $^{**}P < 0.005$ and $^{*}P < 0.05$ using an unpaired 2-tailed Student *t* -test.

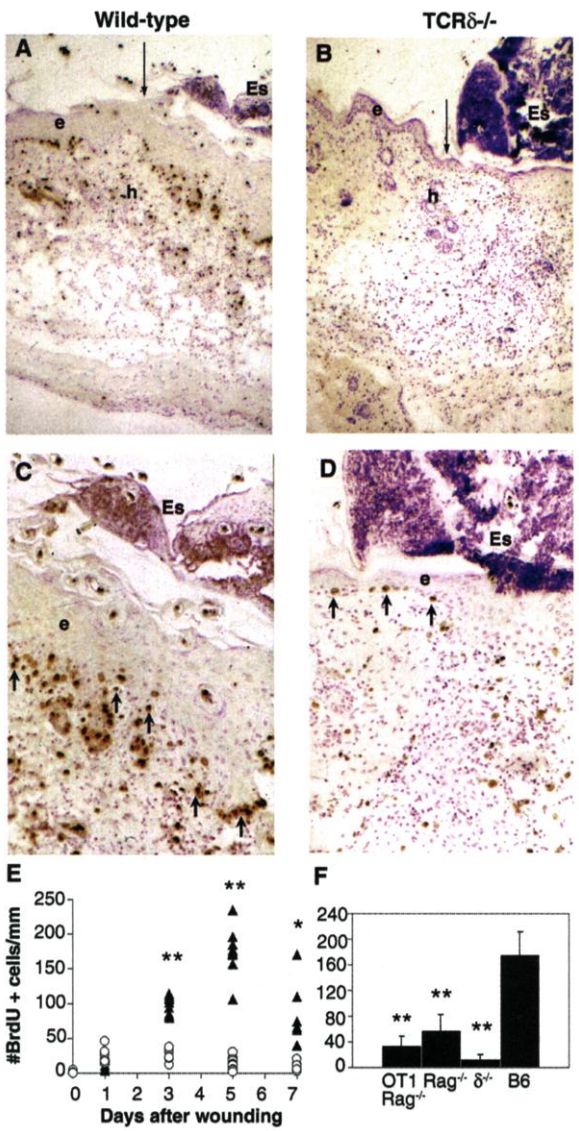

Fig. 2. γδ DETCs affect epidermal thickening and keratinocyte proliferation in wounded tissue. Detection of proliferating cells in wounded tissue by BrdU labeling in wild-type (**A** and **C**) and TCRδ$^{-/-}$ (**B** and **D**) mice 3 days after wounding (*16*). Wound edge is marked with arrow (↓) (A and B) ×100 and (C and D) ×200 magnification. Representative BrdU-positive cells are marked by arrows; e, epidermis; h, hair follicles; and Es, eschar. (**E**) Quantification of BrdU-positive cells in B6 (▲) and TCRδ$^{-/-}$ (○) skin. At each time point, BrdU-positive cells were quantified from at least six individual mice per strain (*16*). (**F**) BrdU incorporation at the wound site 5 days after wounding in wild-type, TCRδ$^{-/-}$, OT-1 Rag$^{-/-}$, and Rag$^{-/-}$ mice. No significant differences were observed between OT-1 Rag$^{-/-}$, Rag$^{-/-}$, and TCRδ$^{-/-}$ mice. $^{**}P < 0.005$ and $^{*}P < 0.01$ using an unpaired 2-tailed Student *t* -test compared to B6 mice.

by a partial loss of the distinctive dendritic shape, although no significant change in DETC density was observed (Fig. 1, B and C). Five days after wounding, the DETCs at the wound margins began to regain their dendritic morphology (*17*). DETCs that were distant from the wound retained their normal shape.

To determine whether DETCs participate in wound repair, wild-type and TCRδ$^{-/-}$ mice received full-thickness wounds in their back skin (*16*), and the rate of wound closure was assessed over a 20-day period. Clear differences in wound size and rate of healing were evident when wild-type and TCRδ$^{-/-}$ wounds were compared on each day after wounding, as shown for day 2 (Fig. 1D). TCRδ$^{-/-}$ mice had a 2- to 3-day delay in wound closure relative to wild-type mice (Fig. 1E). Histological analysis of full-thickness wounds revealed reduced epithelial hyperthickening in the TCRδ$^{-/-}$ mice, relative to wild-type mice (Fig. 2, A through D), suggesting that keratinocyte proliferation was impaired in the absence of γδ DETC. To test this more directly, we injected mice with BrdU at various times after wounding (*16*). Significantly fewer BrdU-positive epidermal cells were detected from the wound edge to the wound center in TCRδ$^{-/-}$ compared to wild-type mice (Fig. 2, C through E). Together, these data indicate that γδ T cells play a role in keratinocyte proliferation and reepithelialization during wound healing.

DETCs are activated through the canonical Vγ3Vδ1 TCR by antigen expressed by stressed keratinocytes. Mice that lack the canonical Vγ3 chain have DETCs with a TCR that retains the original Vγ3 conformation and antigen specificity (*18*), emphasizing the functional relevance of this TCR. To determine if DETC activation in response to wounding requires Vγ3Vδ1, we utilized OT-1 TCR transgenic mice (*19*) on a Rag-1$^{-/-}$ background. In OT-1 mice, all T cells, including DETCs, express an identical Vα2Vβ5 TCR that recognizes an ovalbumin peptide complexed with I-A^{b} (*19*). BrdU incorporation in the wound was diminished (Fig. 2F) and wound closure delayed (*17*) in OT-1 compared to wild-type mice in a manner similar to that observed in TCRδ$^{-/-}$ mice.

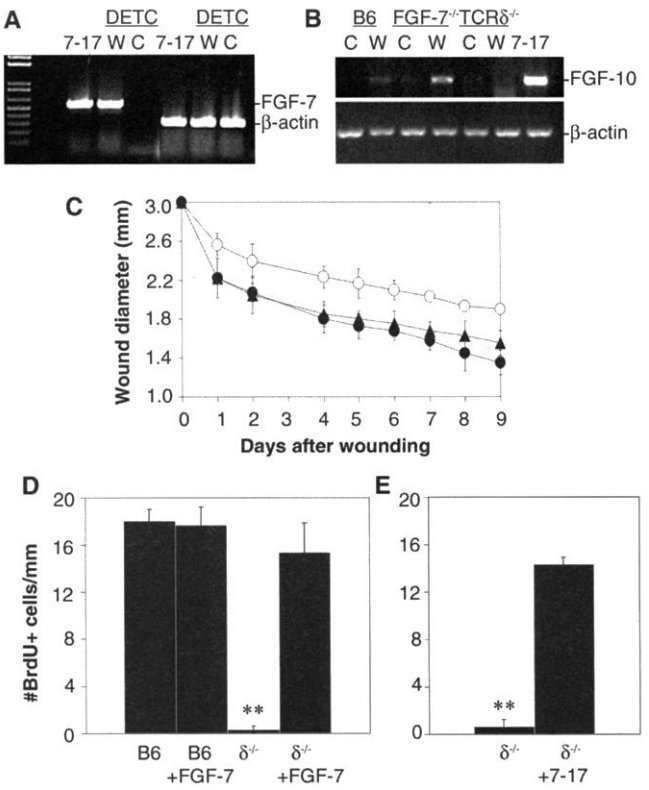

Fig. 3. FGF-7 and FGF-10 mediate the DETC response to wounding. Levels of (**A**) FGF-7 and (**B**) FGF-10 mRNA isolated from purified DETCs from nonwounded (**C**) or day-2-wounded (W) B6 mice, or positive control DETC cell line (7-17) (*16, 29*). Addition of FGF-7 or DETCs to wounds from TCRδ$^{-/-}$ mice in skin organ culture (*24, 25*) restores wound closure and keratinocyte proliferation to wild-type levels. In each experiment, three to four wounds were measured over time for each strain of mouse and culture condition (*16*). (C) Wound closure kinetics in wounded B6 skin (▲), TCRδ$^{-/-}$ skin (○) and TCRδ$^{-/-}$ skin plus FGF-7 (50 μg/ml) (●). BrdU incorporation at the wound site in the presence of (**D**) FGF-7 (50 μg/ml) or (**E**) Con A–activated 7-17 cells. $^{**}P < 0.001$ using an unpaired 2-tailed Student *t* -test. Results in (A) through (E) are representative of two to three experiments.

We conclude that DETCs contribute to wound repair via specific recognition of antigen mediated through the Vγ3Vδ1 TCR.

γδ DETCs do not constitutively produce FGF-7 but require activation through the TCR (*4*). Furthermore, keratinocyte proliferation mediated by γδ DETCs was inhibited by a FGFR2-IIIb neutralizing peptide (*20*), indicating that KGFs are the major epithelial growth factors produced by DETCs (*4*). To determine if γδ DETCs are activated to produce FGF-7 in response to tissue injury, FGF-7 expression was examined in full-thickness wounds (*16*) (Fig. 3A). FGF-7 mRNA was detected in DETCs isolated from the wound area but not from nonwounded skin. In addition, mRNA encoding other factors including TNF-α and IFN-γ was expressed by DETCs following wounding (*17*). The data suggest that DETCs in wounded skin are activated by neighboring, damaged keratinocytes and play a role in wound repair by expressing KGFs and cytokines.

Because no significant wound healing defect has been observed in FGF-7$^{-/-}$ mice (*15, 21*), we examined whether γδ DETCs also produced FGF-10. The importance of FGF-10 in wound healing cannot be studied in FGF-10$^{-/-}$ mice as they die shortly after birth (*22, 23*). Because the DETC cell line 7-17 produced FGF-10 upon TCR stimulation, it was possible that DETCs might participate in wound healing by production of FGF-10. Consistent with this, FGF-10 was detected in wounded skin obtained from wild-type and FGF-7$^{-/-}$ mice, but not in the epidermis of wounded TCRδ$^{-/-}$ mice, indicating that DETCs were the key source of FGF-10 in the wounded epidermis 2 days after wounding (Fig. 3B).

A skin organ culture (SOC) assay was used to further characterize the contribution of γδ DETC-produced factors to wound repair and keratinocyte proliferation (*16, 24, 25*). The in vivo finding that TCRδ$^{-/-}$ mouse skin showed delayed kinetics of wound closure when compared with wild-type mouse skin could be reproduced in the SOC assay (Fig. 3C). Significantly, addition of FGF-7 normalized the wound closure rate and BrdU incorporation observed with TCRδ$^{-/-}$ skin (Fig. 3D). Skin from OT-1 Rag$^{-/-}$ and Rag$^{-/-}$ mice exhibited similar delays in wound closure as TCRδ$^{-/-}$ skin in SOC (*22*). Addition of Con A–activated 7-17 cells to the culture of wounded TCRδ$^{-/-}$ skin restored proliferation to wild-type levels (Fig. 3E). Together, these data indicate that the defect in keratinocyte proliferation and wound closure in TCRδ$^{-/-}$ mice can be attributed to a lack of KGFs produced by DETCs.

Wound healing is a complex process that involves epithelial cell proliferation, granulation tissue deposition, and inflammatory cell recruitment. Many cell types are involved in this process; however, the role of resident DETCs in wound repair has been neglected. Here, we demonstrate that mice lacking DETCs have a significant delay in wound healing and impaired epidermal cell proliferation. We have proposed that DETCs recognize antigen expressed on neighboring epithelial cells following injury or disease (*3*), and participate in tissue repair through the local production of factors including FGF-7 and FGF-10. DETCs are considered a prototype intraepithelial γδ T cell population, raising the possibility that participation in tissue repair is a conserved function shared by intraepithelial γδ T cells in different epithelial tissues. Indeed, we have recently shown that intestinal intraepithelial γδ T cells protect the intestinal mucosa from damage (*26*). Future studies of epithelial diseases such as inflammatory bowel disease, asthma, and wound healing will need to consider the role of intraepithelial γδ T cells in disease progression and tissue repair as well as in design of treatment strategies.

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