Skin-Resident T Cells Sense Ultraviolet Radiation–Induced Injury and Contribute to DNA Repair

Amanda S. MacLeod, Ross Rudolph, Ross Corrden, Ivan Ye, Olivia Garijo and Wendy L. Havran

*J Immunol* published online 7 May 2014
http://www.jimmunol.org/content/early/2014/05/07/jimmunol.1303297

Supplementary Material

http://www.jimmunol.org/content/suppl/2014/05/07/jimmunol.1303297.DCSupplemental

Why *The JI*?

- **Rapid Reviews!** 30 days* from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Skin-Resident T Cells Sense Ultraviolet Radiation–Induced Injury and Contribute to DNA Repair

Amanda S. MacLeod,* Ross Rudolph,†,‡ Ross Corriden,§ Ivan Ye,* Olivia Garijo,* and Wendy L. Havran*

Skin-resident T cells have been shown to play important roles in tissue homeostasis and wound repair, but their role in UV radiation (UVR)–mediated skin injury and subsequent tissue regeneration is less clear. In this study, we demonstrate that acute UVR rapidly activates skin-resident T cells in humans and dendritic epidermal γδ T cells (DETCs) in mice through mechanisms involving the release of ATP from keratinocytes. Following UVR, extracellular ATP leads to an increase in CD69 expression, proliferation, and IL-17 production, and to changes in DETC morphology. Furthermore, we find that the purinergic receptor P2X7 and caspase-1 are necessary for UVR-induced IL-1 production in keratinocytes, which increases IL-17 secretion by DETCs. IL-17, in turn, induces epidermal TNF-related weak inducer of apoptosis and growth arrest and DNA damage–associated gene 45, two molecules linked to the DNA repair response. Finally, we demonstrate that DETCs and human skin-resident T cells limit DNA damage in keratinocytes. Taken together, our findings establish a novel role for skin-resident T cells in the UVR-associated DNA repair response and underscore the importance of skin-resident T cells to overall skin regeneration.

The Journal of Immunology, 2014, 192: 000–000.

Skin-resident T cells (T cells) are particularly numerous in patients taking T cell immunosuppressants for organ transplantation. Dendritic epidermal T cells (DETCs) are necessary for UVR-induced IL-1 production in keratinocytes, which increases IL-17 secretion by DETCs. IL-17, in turn, induces epidermal TNF-related weak inducer of apoptosis and growth arrest and DNA damage–associated gene 45, two molecules linked to the DNA repair response. Finally, we demonstrate that DETCs and human skin-resident T cells limit DNA damage in keratinocytes. Taken together, our findings establish a novel role for skin-resident T cells in the UVR-associated DNA repair response and underscore the importance of skin-resident T cells to overall skin regeneration.

*Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, CA 92037; †Division of Plastic Surgery, Scripps Clinic Torrey Pines, La Jolla, CA 92037; ‡Division of Plastic Surgery, University of California San Diego, La Jolla, CA 92037; and †Division of Pharmacology and Drug Discovery, Department of Pediatrics, University of California San Diego, La Jolla, CA 92093

Received for publication December 9, 2013. Accepted for publication April 10, 2014.

This work was supported by the National Institute of Allergy and Infectious Diseases and the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under Grants R01AI036964 (to W.L.H.), T32AI007244 (to A.S.M.), and K08AR06372901 (to A.S.M.). This is manuscript 25067 from The Scripps Research Institute.

Address correspondence and reprint requests to Dr. Wendy L. Havran, Department of Immunology and Microbial Science, The Scripps Research Institute, 10550 North Torrey Pines Road, IMM-8, La Jolla, CA 92037. E-mail address: havran@scripps.edu

The online version of this article contains supplemental material.

Abbreviations used in this article: CPD, cyclobutane pyrimidine dimer; DETC, dendritic epidermal γδ T cell; eATP, extracellular ATP; GADD45, growth arrest and DNA damage–associated gene 45; γH2AX, phosphorylated form of histone 2A; Nlpr3, NOD-like receptor family, pyrin domain–containing 3; rh, recombinant human; SCC, squamous cell carcinoma; TWEAK, TNF-related weak inducer of apoptosis; UVR, UV radiation; WT, wild-type.

Copyright © 2014 by The American Association of Immunologists, Inc. 0022-1767/13/$16.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1303297

Published May 7, 2014, doi:10.4049/jimmunol.1303297

The Journal of Immunology
IL-17, in turn, upregulates epidermal TNF-related weak inducer of apoptosis (TWEAK) and growth arrest and DNA damage–associated gene 45 (GADD45), two genes with known functions in DNA repair (18, 19). We furthermore demonstrate that human skin-resident T cells and DETCs play a critical role in limiting UVR-induced DNA damage–associated phosphorylated form of histone 2A (γH2AX) and cyclobutane pyrimidine dimer (CPD) formation in keratinocytes. Taken together, this study identifies a previously unknown role of skin-resident T cells in sensing solar injury and potentiating the keratinocyte DNA repair response. Our findings indicate that the eATP pathway could be therapeutically targeted to alter susceptibility or treat UV-induced skin cancer and may offer an alternative to phototherapy.

Materials and Methods

Human skin samples, cell preparation, and stimulation

This study was approved by the Scripps Investigational Review Board. Normal skin samples were obtained from otherwise discarded tissue from plastic surgery procedures performed at Scripps Green Hospital (La Jolla, CA) and Scripps Clinic Ambulatory Surgical Center Carmel Valley (San Diego, CA). Tissue samples were used to perform skin organ cultures or to obtain skin-resident T cells as previously described (2, 3) with the exception that cells were kept in complete RPMI 1640 medium with 10% FCS without cytokines or, for experiments where T cells supernatants were used, were stimulated in Epilife medium (Cascade Biologies). Anti-CD3 (OKT3, Sigma-Aldrich) Abs were dilute in ELISA coating buffer (0.05 mM Tris, 150 mM NaCl [pH 8.0]) and immobilized to individual wells of 96-well flat-bottom microtiter ELISA plates (Immunol). Approximately 0.5 × 10^6 skin-resident T cells/ml were stimulated with plate-bound anti-CD3 Abs (OKT-3), ATP (2 mM; Sigma-Aldrich), or p2X7 (15 mM; Sigma-Aldrich), keratinocyte-conditioned medium (described below), or combinations thereof, as stated in the figure legends. Skin organ cultures from newborn donors were stimulated for 24 h with recombinant human IL-17 (200 ng/ml; R&D Systems), skin-resident T cell supernatants, or were irradiated with an EB-280C/12 UVB lamp (200 mJ/cm^2; Spectroline, Edison, NJ), predominate emission 312 nm, 270–390nm emission range) before immunofluorescence staining was performed. Normal human keratinocytes were purchased from Cascade Biologies and were grown in serum-free Epilife cell culture medium containing 0.06 mM Ca^2+. Epilife defined growth supplement, 50 U/ml penicillin, and 50 mg/ml streptomycin. Keratinocyte cultures from healthy donors were stimulated for 24 h with recombinant human IL-17 (200 ng/ml; R&D Systems), skin-resident T cell supernatants, or were irradiated with an EB-280C/12 UVB lamp (200 mJ/cm^2; Spectroline, Edison, NJ) and immobilized to individual wells of 96-well flat-bottom microtiter ELISA plates (Immunol). Approximately 0.5 × 10^6 skin-resident T cells/ml were stimulated with plate-bound anti-CD3 Abs (OKT-3), ATP (2 mM; Sigma-Aldrich), or p2X7 (15 mM; Sigma-Aldrich), keratinocyte-conditioned medium (described below), or combinations thereof, as stated in the figure legends. Skin organ cultures from healthy donors were stimulated for 24 h with recombinant human IL-17 (200 ng/ml; R&D Systems), skin-resident T cell supernatants, or were irradiated with an EB-280C/12 UVB lamp (200 mJ/cm^2; Spectroline, Edison, NJ), predominate emission 312 nm, 270–390nm emission range) before immunofluorescence staining was performed. Normal human keratinocytes were purchased from Cascade Biologies and were grown in serum-free Epilife cell culture medium containing 0.06 mM Ca^2+. Epilife defined growth supplement, 50 U/ml penicillin, and 50 mg/ml streptomycin. Keratinocyte cultures were maintained for up to five passages. Keratinocytes were used at 75–80% confluence and were stimulated in 6- to 12-well plates (Corning) with rhIL-17, rhIL-1, or rhIFN-γ (R&D Systems) for subsequent RNA isolation or were stimulated in 2- to 4-well chamber slides (Lab-Tek) with cell-free supernatants from the anti-CD3–activated skin-resident T cells (T cell supernatants) and were irradiated with 15 mJ/cm^2 UVB before immunohistochemical analyses. For some experiments, cell culture medium was collected from human keratinocytes, called keratinocyte-conditioned medium, were collected from keratinocytes within 30–60 min following irradiation with UVB (15 mJ/cm^2) or without.

Flow cytometry and FACS

Abs and appropriate IgG controls were conjugated to FITC, PE, PerCP-Cy5.5, PE-Cy7, Pacific Blue, or allophycocyanin. Abs to Vy3 (536), TCRβ (H57-597), CD4 (GK1.5), and pan-CD8 (53–2–9) were purchased from BioLegend. The Abs TCRε (53–22), CD3 (HT3 17A2, and 145-2, 2C11), and CD45 (H30) Abs were purchased from BioLegend. Abs to IL-17 (ebio64DEC17), CD8 (53-5.8), and CD69 (H12.2F3) were purchased from eBioscience. Ab to P2X1 receptor was purchased from Alomone Laboratory. For detection of intracellular IL-17, human skin-resident T cells were stimulated with keratinocyte-conditioned medium for 18 h in the presence or absence of interleukin-1β (15 U/ml) and were restimulated with PMA (2 ng/ml) and ionomycin (1 μM) for an additional 3 h in the presence of brefeldin A (5 μM/ml) and monensin (1:1000; eBioscience) before cells were fixed and permeabilized with Cytofix/Cytopermin reagents from BD Biosciences. Cells were acquired with DiVa 5.0 software on a digital LSRII and analyzed with FlowJo software (Tree Star). For some experiments, DETCs were purified by FACS-sorting based on Vy3^+ TCRε^+ P2X7^+ CD3^+CD45^- and keratinocytes were sorted from epidermal cell suspensions being CD45^-CD3^+; the purity of sorted cell populations was between 91 and 99%.

Proliferation, ELISA, and bioluminescence assays

For proliferation assays and ELISA, ~0.5–1 × 10^6 cells/ml were stimulated as stated in the figure legends. For proliferation assays, DETCs or human skin-resident T cells were stimulated for 18–24 h after addition of 0.5 μCi/ml [3H]thymidine (MP Biomedical) Samples were harvested 18–24 h later, and [3H]thymidine incorporation was measured using a Beckman LS181 scintillation counter (Beckman Coulter). For analyses of secreted cytokines, supernatants were removed at various time points and immediately stored at −20°C until use. Supernatants from DETCs were analyzed for presence of IL-17A by ELISA (eBioscience). Bioluminescence-based ATP measurements (Roche) were performed on supernatants collected from UVR-treated or nontreated skin organ cultures.

RNA isolation and RT-PCR

Total RNA was isolated from cells using an RNAeasy Micro kit (Qiagen) or from tissue using the TRIzol reagent (Invitrogen). RNA was reverse-transcribed using the iScript cDNA synthesis kit (Bio-Rad), and resulting cDNA was amplified using FastStart Universal SYBR Green Master Mix (Roche). Primers for amplification were used as previously described (13). Fold induction of gene expression was normalized to β-actin and calculated using the 2^(-ΔΔCt) method.

Epidermal sheet and skin immunofluorescence

Epidermal ear sheets from mice were stained with PE-conjugated Abs to Vy3 and DAPI (Sigma-Aldrich) as previously described (13). Quantification of DETCs numbers was performed using ImageJ software. For histological analysis of γH2AX (Active Motif), CPD (Kamiya Biomedical), and TWEAK (CARL-1, Abd Serotec), tissue was methanol- or paraformaldehyde-fixed and incubated in a blocking solution of PBS containing 2.5% normal goat serum (Jackson ImmunoResearch Laboratories), 2.5% normal donkey serum (Jackson ImmunoResearch Laboratories), 1% BSA (Calbiochem), 2% fish gelatin (Sigma-Aldrich), and 0.1% Triton X-100 at room temperature before incubation with primary Abs or appropriate IgG controls overnight at 4°C. Secondary Abs were FITC-, PE-, or Cy3-conjugated (Jackson ImmunoResearch Laboratories). After subsequent
wearing, sections were mounted with ProLong Gold antifade containing DAPI (Invitrogen). Sections were visualized using a Nikon Eclipse E800 microscope and digital images were acquired with a Zeiss AxioCam HRc camera.

Statistical analyses

Data are presented as means; error bars are SEM. Data shown are representative of at least three independent experiments unless otherwise indicated. Statistical significance was measured using a two-tailed Student t test. A p value of <0.05 was considered significant.

Results

Soluble factors released from UV-irradiated keratinocytes activate DETCs in mice

UVR is a common environmental hazard of the skin; however, its role in skin-resident T cell immunology is not well understood. First, we investigated whether exposure to UVR activates DETCs in wild-type (WT) mouse skin. Similar to other tissue-resident T cells, DETCs are present in a semiactivated state and constitutively express CD69 (2, 8, 11, 20). Once activated, DETCs upregulate CD69 expression and change their cellular morphology from a dendritic to a circular cell shape (7, 21). Following in vivo UVR treatment, we observed an increase in CD69 expression on DETCs (Fig. 1A). DETC rounding began within 30 min following UVR, and by 3 h most DETCs had a rounded phenotype that was retained for at least 18 h after UVR (Fig. 1B, 1C). These findings suggested that acute UVR leads to DETC activation.

Soluble factors released from epithelial cells have been shown to modulate T cell responses (22–25), and therefore we next assessed the capacity of cell-free supernatants from UVR-treated keratinocytes to induce DETC proliferation (Fig. 2A). Supernatants collected from UVR-treated or mock-treated keratinocytes 30 min after UVR exposure increased DETC proliferation in the presence but not in the absence of TCR stimulation (Fig. 2A and data not shown), suggesting that a soluble factor from keratinocytes is rapidly released upon UVR and enhances TCR-mediated DETC activation. Expanding the previous finding of increased ATP release from keratinocytes upon UVR (17), we detected increased levels of eATP in supernatants from UVR-treated skin organ cultures (Fig. 2B), indicating a possible role for eATP in the acute UV response. This idea was supported by three observations. First, enzymatic hydrolyzation of ATP by apyrase inhibited the capacity of supernatants from UVR-treated keratinocytes to increase DETC proliferation (Fig. 2A). Second, apyrase treatment of UVR-treated skin organ cultures blocked the UVR-induced increase in CD69 expression on DETCs (Fig. 2C). Third, exogenous ATP was sufficient to mediate DETC activation in situ, as eATP alone stimulated DETC rounding, visualized by immunofluorescence staining of epidermal ear sheets with Abs recognizing the gd T cells (7, 21). Taken together, these findings strongly suggested a previously unrecognized role for UVR-induced eATP in regulating DETC activation.

Murine epidermal cell populations express distinct ATP-sensing P2 receptors

Extracellular ATP signaling is mediated through membrane-bound purinoreceptors and is implicated in the regulation of both innate and adaptive immune responses (15, 26, 27). Among the studied P2X receptors, P2X1, P2X4, and P2X7 receptors have been previously described to be expressed by peripheral gd T cells (28). However, the presence of purinoreceptors on DETCs and the functional role of eATP signaling for DETC biology are unknown. Analyses of epidermal cell populations revealed that DETCs and keratinocytes express distinct P2 receptors (Fig. 3), suggesting that eATP serves multiple functions in the skin, in line with previous reports (27). The mRNAs encoding the ionotropic P2X1, P2X2, P2X3, and P2X5 and the metabotropic P2Y6 and P2Y12 receptors are expressed at higher levels by DETCs compared with keratinocytes (Fig. 3). In contrast, P2X4, P2X7, and P2Y1 receptors are expressed at higher levels by keratinocytes compared with DETCs. These results suggested that epidermal cells express several distinct purinoreceptors.

Dual functions of extracellular ATP for murine DETC activation

While under steady-state conditions, eATP levels are low and transient eATP increases desensitization of distinct purinoreceptors, whereas upon cell stress or cell burst, eATP concentrations rise rapidly and can activate purinoreceptors with even low binding constants to eATP, such as the P2X7 receptor. Our
the presence or absence of apyrase (gated on live Thy1.2+Vγ69 on DETCs 6 h following UVR treatment of skin organ cultures in an ATP-dependent manner. Representative flow cytometric analyses of error bars indicate SEM. (C) ATP stimulation. Ear sheets were treated with 2 mM ATP for 3 h and DETCs are shown). (D) DETCs change their cellular morphology following ATP stimulation. Ear sheets were treated with 2 mM ATP for 3 h and DETC morphology was evaluated as described in Fig. 1B. Original magnification ×200 and ×400 (insets). *p ≤ 0.05, **p ≤ 0.01 by two-tailed Student t test.

Laboratory has recently shown that a subset of DETCs produces IL-17A following acute skin injury to promote wound repair (13). Because eATP was found to be increased in UV-irradiated mouse skin (Fig. 2B), a form of skin injury, and DETCs are shown to readily express multiple P2X and P2Y receptors (Fig. 3), we hypothesized that eATP may modulate IL-17 production by DETCs. When pure populations of Vγ3+ DETCs were stimulated with eATP in the presence of low concentrations of immobilized anti-CD3, DETCs produced significantly more IL-17 than when stimulated with low concentrations of immobilized anti-CD3 alone. The response of DETCs to eATP could be blocked by the preincubation of ATP with apyrase (Fig. 4A), whereas addition of apyrase alone or eATP alone did not alter IL-17 production (Fig. 4A and data not shown).

We then sought to test whether conditioned culture medium from UVR-treated keratinocytes was sufficient to increase IL-17 secretion by DETCs. Indeed, stimulation of WT DETCs with conditioned medium from UVR-treated but not mock-treated WT keratinocytes increased IL-17 production by WT DETCs in the presence of low concentrations of immobilized anti-CD3 (Fig. 4B). Apyrase inhibited the capacity of keratinocyte-conditioned medium from UVR-treated WT keratinocytes to induce IL-17 secretion by WT DETCs (Fig. 4B). These results suggested that UVR-treated keratinocytes can stimulate WT DETC cytokine production through an ATP-dependent mechanism.

Keratinocytes are a rich source of IL-1α and, to a lesser extent, IL-1β. Both IL-1α and IL-1β have been shown to play roles in modulating IL-17 production by WT DETCs. Previous studies from our laboratory have demonstrated that recombinant IL-1β together with IL-23 increases IL-17 production by WT DETCs in the presence of TCR stimulation (13). We therefore tested the possibility that UVR-induced IL-1 release from keratinocytes affects WT DETC IL-17 production. The biological activity of IL-1α and IL-1β can be simultaneously blocked by antagonizing the IL-1R. When anakinra, a clinically used recombinant, nonglycosylated form of the human IL-1R antagonist, was added to WT DETC cultures during stimulation with supernatants from UVR-treated keratinocytes, IL-17 secretion by DETCs was significantly decreased (Fig. 4B).

IL-1β secretion is mediated by a pathway involving NOD-like receptor family, pyrin domain-containing 3 (Nlrp3) inflammasome and caspase-1 activation, following binding of eATP to the P2x7 receptor, whereas IL-1α secretion is at least partially dependent on IL-1β secretion (29–32). Compared to other members of the P2

![FIGURE 2](http://www.jimmunol.org/figs/1284293/fig2.jpg)

**FIGURE 2.** UV-irradiated keratinocytes activate murine DETCs in an ATP-dependent manner. (A) UV-induced ATP release from keratinocytes promotes DETC proliferation in the presence of anti-CD3 stimulation. Proliferation was measured in triplicates by [3H]thymidine incorporation in DETCs 48 h following stimulation with conditioned medium collected from keratinocytes that were either UVR-treated (Kerat. cond. med. (UV)) or untreated (Kerat. cond. med. (no UV)) in the presence or absence of apyrase. Culture medium alone and apyrase alone were included as controls and did not stimulate DETC proliferation (data not shown). Data are presented as means from triplicates; error bars indicate SEM. (B) Measurement of ATP in the supernatants from mock- and UVR-treated skin organ cultures by bioluminescence. Data are presented as means (n = 5); error bars indicate SEM. (C) UVR-induced CD69 expression on DETCs is mediated via extracellular ATP. Representative flow cytometric analyses of CD69 on DETCs 6 h following UVR treatment of skin organ cultures in the presence or absence of apyrase (gated on live Thy1.2+Vγ3+). Gray shaded graph indicates IgG control for non-UVR-treated skin and was similar to UVR-treated control IgG or apyrase-treated control IgGs (data not shown). (D) DETCs change their cellular morphology following ATP stimulation. Ear sheets were treated with 2 mM ATP for 3 h and DETC morphology was evaluated as described in Fig. 1B. Original magnification ×200 and ×400 (insets). *p ≤ 0.05, **p ≤ 0.01 by two-tailed Student t test. Data are presented as means from triplicates; error bars indicate SEM. (Exogenous ATP in the presence or absence of apyrase. Data are presented as means from triplicates; error bars indicate SEM. **p ≤ 0.05, ***p ≤ 0.01 by two-tailed Student t test.

![FIGURE 3](http://www.jimmunol.org/figs/1284293/fig3.jpg)

**FIGURE 3.** Distinct expression of purinoreceptors in murine keratinocytes and DETCs. Representative real-time quantitative PCR analysis of various P2X and P2Y receptors in FACS-sorted populations of DETCs and epidermal keratinocytes from mouse skin is shown.

![FIGURE 4](http://www.jimmunol.org/figs/1284293/fig4.jpg)

**FIGURE 4.** ATP increases IL-17 production by murine DETCs via direct and indirect mechanisms. (A) IL-17 production by FACS-sorted populations of anti-CD3-stimulated WT DETCs 24 h after addition of exogenous ATP in the presence or absence of apyrase. Data are presented as means from triplicates; error bars indicate SEM. (B) IL-17 production by WT DETCs 24 h following stimulation with conditioned medium collected from UVR-treated (Kerat. cond. med. (UV)) or nontreated (Kerat. cond. med. (no UV)) keratinocytes in the presence or absence of apyrase or the IL-1R antagonist anakinra. (C) Conditioned medium from UVR-treated WT keratinocytes induces significantly more IL-17 secretion by anti-CD3-activated WT DETCs 24 h following stimulation than conditioned medium from P2x7−/− and Casp1−/− UVR-treated keratinocytes. Data are presented as means from triplicates; error bars indicate SEM. *p ≤ 0.05, **p ≤ 0.01 by two-tailed Student t test.
receptor family, P2X7 receptor has only a low binding constant to eATP, acting especially in response to high eATP levels and is therefore considered a danger signaling receptor (33, 34). In contrast to DETCs, keratinocytes express the P2X7 receptor (Fig. 3) (33, 34), as well as all components for Nlrp3 inflammation assembly (31, 33, 34). To test the possibility that activation of P2X7 receptors by UVR-induced ATP release from keratinocytes induces IL-1 to stimulate IL-17 production by WT DETCs, conditioned medium from UVR-treated WT, P2x7^−/−, or Casp1^−/− keratinocytes was added to WT DETC cultures. Increased IL-17 production by WT DETCs was observed only when conditioned medium from WT keratinocytes was added to DETC cultures, whereas conditioned medium from UVR-treated P2x7^−/− or Casp1^−/− keratinocytes had minor effects on IL-17 production by WT DETCs (Fig. 4C). In concert with the finding that secretion of IL-1β and to a far lesser extent also IL-1α was diminished in P2x7^−/− keratinocytes upon UV treatment (Supplemental Fig. 1), our findings suggest that UVR induces ATP-mediated IL-1 release from keratinocytes, which increases IL-17 production by WT DETCs. Taken together, these results highlight that both direct and indirect mechanisms may account for eATP-mediated DETC activation and IL-17 production.

**WT but not Tcrd^−/− mice are protected from UVR-induced DNA damage-associated\(\gamma\)H2AX and CFD formation**

Keratinocytes are sensitive targets of UVR, which causes DNA damage characterized by DNA double-strand break, \(\gamma\)H2AX, and CFD formation. When proper DNA repair fails, cells may undergo cellular proliferation and oncogenic development. Tissue-resident T cells, such as DETCs, provide local surveillance functions (35–39). Therefore, we hypothesized that following UVR-induced activation, DETCs may initiate a protective response to limit UV damage to keratinocytes early before skin carcinogenesis can evolve. To test this hypothesis, we assessed the DNA damage response in keratinocytes 3, 24, and 48 h following UVR treatment. Cells positive for \(\gamma\)H2AX, a marker of double-strand break damage (39), as well as CFP^+ cells were observed with similar frequency in skin of WT and Tcrd^−/− mice at 3 h following UVR (Fig. 5A–C), indicating that equal DNA damage occurred. In contrast, at 24 or 48 h following UVR, a higher frequency of \(\gamma\)H2AX^+ and CFP^+ cells was observed in UV-exposed skin from Tcrd^−/− compared with WT mice (Fig. 5A–C). Taking together, these results suggested that DNA repair of UV-induced lesions is reduced in the absence of \(\gamma\)6 T cells in the skin of mice. To further demonstrate that UV-induced eATP could initiate an epidermal response aimed at limiting DNA damage, we also tested whether eATP reduces the frequency of CFD formation in UVR-treated skin. Pretreatment of epidermal sheets with eATP prior to UVR resulted in only a minor reduction of CFD formation measured at 24 h, whereas pretreatment with apyrase enhanced the frequency of CFP^+ cells following UV- compared with eATP-treated epidermal ear sheets, but the increase was not significant compared with nontreated ear sheets (Supplemental Fig. 2).

**GADD45** is a major participant in genomic stability and DNA repair (19, 36, 38, 40). It exhibits low constitutive expression, is predominantly intranuclear, and becomes transcriptionally activated by UVR, hyperoxia, and endotoxin (37, 41, 42). The relevance of GADD45 to the UV protection response is supported by previous observations that GADD45 is essential for protection against UV-induced skin cancerogenesis (19). TWEAK is a soluble protein known to bind to the TWEAK receptor, the fibroblast growth factor-inducible 14 receptor, and has been previously shown to induce Gadd45 (18). In the present study, we show that Gadd45 and Tweak are upregulated upon in vivo UVR treatment in the epidermis from WT mice (Fig. 5D, 5E). In contrast, Tcrd^−/−, Il17a^−/−, and Rag2^−/− mice showed impaired upregulation of Gadd45 and Tweak following UVR (Fig. 5D, 5E), demonstrating that DETCs and IL-17 play critical roles in the regulation of Gadd45 and Tweak expression in the skin. Thus, the lack of GADD45 and TWEAK in Tcrd^−/− skin following UVR may contribute to defective keratinocyte DNA repair, as demonstrated by the higher frequency of \(\gamma\)H2AX^+ and CFP^+ keratinocytes in Tcrd^−/− mice.

**Human skin-resident T cells contribute to the UVR response**

Identification of the protective function of DETCs in the UVR response in mice and the critical role of human skin-resident T cells in skin immunity raised the possibility that human skin-resident T cells also sense solar injury. To assess whether the eATP/skin-resident T cell axis is active in human skin, we first examined whether human skin-resident T cells respond to eATP. Increased proliferation was observed in human skin-resident T cell cultures following eATP stimulation, independent of the presence of anti-CD3 stimulation (Fig. 6A). Several mRNAs of purinergic receptors, including those encoding P2X1, P2X4, and P2X7 receptors, have been recently identified in human T cells (28). Because P2X1 receptor expression but not P2X4 or P2X7 receptor expression was high in murine DETCs (Fig. 3), we focused on the human P2X1 receptor, and we demonstrated that it is detected on activated human skin-resident T cells (Supplemental Fig. 3A, 3B). Stimulation of human skin-resident T cells with conditioned medium from normal human keratinocytes that were subjected to UVR allowed further investigation of the involvement of
keratinocyte-derived ATP in skin-resident T cell activation. IL-17 production in skin-resident T cells was observed following stimulation with conditioned medium from UVR-treated keratinocytes (Fig. 6B). The absolute frequency of IL-17–producing skin-resident T cells did not dramatically change between stimulation with conditioned medium from UVR-treated keratinocytes and conditioned medium from non-UVR–treated keratinocytes; however, UVR-treated keratinocyte supernatants increased the proportion of T cells producing high amounts of IL-17 (Fig. 6B). This subset of IL-17hi producers was sensitive to ATPase treatment (Fig. 6B). Furthermore, rhIL-17 upregulated GADD45A and TWEAK mRNA levels in human cultured keratinocytes, whereas rhIL-4 exerted an effect only on GADD45, and IFN-γ had minor effects on GADD45 and TWEAK (Fig. 6C). Increased TWEAK immunoreactivity was also observed in human skin organ cultures treated with rhIL-17 and was localized to the epidermal layer, suggesting that keratinocytes are the major cell type in the skin to upregulate TWEAK upon IL-17 stimulation (Fig. 6D). Finally, supernatants from activated skin-resident T cells reduced the frequency of γH2AX+ cells in human UV-treated skin (Supplemental Fig. 3C). To confirm that it was UVR-induced DNA damage present in keratinocytes that was blocked by skin-resident T cell supernatants, cultured keratinocyte monolayers were analyzed for γH2AX immunoreactivity (43) following UVR treatment (Fig. 6E). UVR-treated keratinocytes showed a higher frequency of γH2AX+ keratinocytes than did those incubated with skin-resident T cell supernatants (Fig. 6E, 6F). Taken together, these results show a novel function of human skin-resident T cells in providing protection for keratinocytes against UVR-induced DNA damage.

Discussion

UVR-induced DNA damage has been causatively linked to many skin cancers, including SCCs (1, 16, 44). An intact T cell immune system is essential to maintain tissue surveillance and prevent skin carcinogenesis (5, 16). In fact, T cell immunosuppression bears a high risk for cutaneous SCC development, and skin-resident T cell numbers are critically reduced in human cutaneous SCC lesions, supporting the idea that impaired function or loss of this protective T cell population in the skin may be associated with SCC development. However, the role of skin-resident T cells in the acute response to cutaneous UVR has not been well studied. Because skin-resident T cells play critical roles in cutaneous immunity, we examined the status of these sentinel cells in UVR-irradiated skin of humans and mice and found that these cells become activated upon UVR exposure and play a novel and yet unrecognized role in the epidermal DNA repair response.

UVR leads to skin-resident T cell activation through a mechanism involving eATP, a danger signaling molecule. Our study demonstrates that release of ATP from UVR-treated keratinocytes results in autocrine and paracrine immune responses, ultimately promoting skin-resident T cell activation. The mechanisms by which eATP affects DETC activation may occur on multiple levels, including cell morphological changes, proliferation, Ca2+ influx, and effects on neighboring keratinocytes (summarized in Supplemental Fig. 4). Interestingly, activation of the P2X1 receptor has been previously linked to changes in cell shapes and Ca2+ influx (45), whereas P2X7 receptor activation has been previously linked to inflammasome-mediated IL-1 secretion (46). In line with the latter observation, we found that IL-17 production in skin-resident T cells is enhanced by UVR-induced IL-1 release from keratinocytes. Furthermore, we demonstrate that eATP enhances TCR-mediated DETC activation, but is ineffective in the absence of TCR stimulation. This result is in line with previous findings from our laboratory showing that DETCs require TCR stimulation for optimal activation (13). Hence, knowledge about the nature of the yet unidentified DETC TCR ligand will help to further delineate DETC biology.

ATPase treatment or IL-1R blockade diminished the production of IL-17 by WT DETCs when stimulated with UVR-treated WT keratinocyte supernatants. This important finding is consistent with observations from this work and others that UVR induces IL-1 secretion, and that WT keratinocytes have the cellular machinery to orchestrate P2X7 receptor signaling, inflammasome assembly, and subsequent IL-1 maturation and processing (29, 46). Our findings expand these observations by directly showing a link between UVR, ATP release, keratinocyte activation, IL-1 release, and DETC IL-17 production (summarized in Supplemental Fig. 4). We cannot exclude the possibility that eATP may also activate other epidermal cells, such as Langerhans cells in vivo, which
express multiple purinoreceptors as well (A.S. MacLeod, unpublished observations and Ref. 47), to provide additional IL-1 (48), but may also contribute through other ATP-dependent biological effects to the cutaneous UVR response in vivo (49). Interestingly, treatment of mouse skin with a P2X7 receptor ligand has been shown to inhibit formation of skin papillomas and carcinomas in the murine two-stage carcinogenesis model (50), suggesting a protective role for P2X7 receptor activation. Additionally, our results on IL-1R blockade further support clinical precaution advisories stating that, while taking the IL-1R antagonist anakinra, patients should practice enhanced skin cancer prevention, that is, sun protection, as ~9% of patients under anakinra therapy have been reported to develop skin cancers (51). Taken together, our data strongly support that eATP signaling in the skin is an important pathway for DETC activation following UVR exposure and may be important to alarm the immune system about solar skin damage.

We demonstrate that human skin-resident T cells were activated by eATP; however, in contrast to DETCs, they did not require concomitant stimulation through the TCR. For our studies, human skin-resident T cell explant cultures were used. It is possible that explant cultures may contain other skin cells that contribute to human skin-resident T cell activation and that may explain this difference. Nonetheless, our results suggest that eATP-mediated signals are critical for both DETCs and human skin-resident T cell activation.

Our findings may have multiple implications for human health. SCCs comprise epithelial-derived cancers of the skin, lung, esophagus, urinary bladder, prostate, lung, and cervix and often develop over many years through a multistep process, including initiation, proliferation, and progression, and they are accompanied by local or systemic immunosuppression (52, 53). Cutaneous SCCs are frequently caused by excessive UV irradiation, and UVR-induced DNA-damaged keratinocytes bear the risk to eventually develop into carcinomas. We find that skin-resident T cells contribute to the DNA repair response in keratinocytes upon acute UVR exposure, supporting their critical role in skin homeostasis and surveillance function. Thus, our findings raise the possibility that skin-resident T cells may be involved in the very early control of DNA damage in keratinocytes to protect from skin cancer development. This newly discovered role for skin-resident T cells may explain previous clinical reports that DETC-derived cytokines may modulate additional repair mechanisms, such as nucleotide excision repair enzymes, to enhance DNA repair, similar to previous observations made for IL-12 and other cytokines (59).

Knowledge of the eATP/skin-resident T cell axis may have major implications for the development of therapeutic targets to improve UVR-induced skin damage, enhance our understanding of the use of phototherapy to ameliorate disease states, such as psoriasis, eczema, or mycosis fungoides, and shed light on ATP-mediated immunity in T cell and epithelial cell biology.

**Acknowledgments**

We thank Drs. Y. Iwakura and K. L. for providing Il17a−/− mice, Drs. R. Flavell and R. Ulevitch for providing Casp1−/− mice, Dr. C. Suh for providing Rag2−/− mice, and Dr. K. Mowen for providing P2x7−/− mice. We thank Drs. R.L. Gallo, G. Sen, D. Mistry, C. Conche, L. Sternberg, K. Sauer, V. Rybakin, N. Gascoigne, J. Teijaro, S. Arandjelovic, M.N. Boddy, and H. Hoffman for reagents and advice. We thank Drs. D.A. Witherden, M. Chabod, and H. Hoffman for advice and critical reading of the manuscript.

**Disclosures**

The authors have no financial conflicts of interest.

**References**


