its low cost and noninvasive nature, STS allows for the collection of samples from large patient cohorts and can be useful for disease endotyping, as collected STS can be readily used for lipid, protein, and RNA analysis. (5) STS procedure can be useful for skin sampling in various age groups, including infants (Kim et al., 2016; McAleer et al., 2019).

In conclusion, STS could be a good standard procedure to evaluate epidermal differentiation markers from the SC and the upper granular layer of the epidermis. Moreover, this method is a safe and reliable strategy to evaluate cellular and molecular characteristics of the skin barrier in both children and adults.

Data availability statement
No datasets were generated or analyzed during this current study.

CONFLICT OF INTEREST
The authors state no conflict of interest.

ACKNOWLEDGMENTS
This study was supported by National Institutes of Health grants: AR41256, UL1TR002535, and UL1TR002535. Additionally, the authors wish to acknowledge the Edelstein Family Foundation for its generous support of this work.

AUTHOR CONTRIBUTIONS
Conceptualization: BEK, DYML; Data Curation: CB, PT; Formal Analysis: BEK, EG, DYML; Investigation: PSK, KN; Writing - Original Draft Preparation: BEK, EG, DYML; Writing - Review and Editing: BEK, PSK, KN, CB, PT, DYML

Byung Eui Kim1, Elena Goleva1, Peter S. Kim1, Kathryn Norquest1, Caroline Bronchick2, Patricia Taylor2 and Donald Y.M. Leung1,*

1. Department of Pediatrics, National Jewish Health, Denver, Colorado; and 2. Clinical Translational Research Center, National Jewish Health, Denver, Colorado
*Corresponding author e-mail: Leungd@njhealth.org

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2019.03.1160.

REFERENCES


TO THE EDITOR
Inflammasomes are multiprotein complexes that respond to infection or injury to activate inflammation. Inflammatory caspases, caspase-1, -4, and -5 in humans, and their murine orthologues caspase-1 and -11, are crucial components of inflammasomes, responsible for the maturation and secretion of IL-1β and IL-18 and for pyroptosis (inflammatory cell death) (Creagh, 2014). Psoriasis is a chronic inflammatory skin condition with a range of clinical manifestations. The most common manifestation is chronic plaque psoriasis, where the adaptive immune response predominates. However, innate and autoimmune inflammatory events, governed by IL-1β (Martinez-Quiles and Goldbach-Mansky, 2018), prevail in pustular forms of psoriasis (Liang et al., 2017).

Caspase-11—Mediated Cell Death Contributes to the Pathogenesis of Imiquimod-Induced Psoriasis

Journal of Investigative Dermatology (2019) 139, 2389–2393; doi:10.1016/j.jid.2019.05.010

Abbreviation: IMQ, imiquimod

Accepted manuscript published online 5 June 2019; corrected proof published online 16 August 2019
© 2019 The Authors. Published by Elsevier, Inc. on behalf of the Society for Investigative Dermatology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Figure 1. Reduced features of IMQ-induced psoriasis in Casp-11–/– mice. Aldara (50 mg) was applied to the back of shaved Casp-11+/+ and Casp-11–/– mice for 4 days, consecutively. (a) Skin was photographed on day 4. (b) Scaling and (c) erythema were scored daily (scale 0–4: 0, none; 1, slight; 2, moderate; 3, marked; 4, very marked). 5 μm FFPE skin sections were probed for (d) PCNA and (e) epidermal positivity was quantified (PCNA positive cells/field). Skin sections were probed and counted for (f, g) CD11b and (h, i) endomucin. Relative (j) Ang-2 and (k) TSP-1 mRNA expression in digested skin was determined by qPCR. Control (n = 2) and Aldara (n = 5), error bars represent mean ± SEM. Data is representative of three independent experiments. Two-way ANOVA, followed by Bonferroni post-test, was used to analyze statistical significance between Casp-11+/+ and Casp-11–/– groups. *P < 0.05, **P < 0.01, ***P < 0.001. ANOVA, analysis of variance; FFPE, formalin-fixed, paraffin-embedded; IMQ, imiquimod; PCNA, proliferating cell nuclear antigen; qPCR, quantitative PCR; SEM, standard error of the mean. 10× magnification, Bar = 20 μm.
Figure 2. Caspase-11-mediated pyroptosis contributes to the inflammatory phenotype of IMQ-induced psoriasis. Skin sections from 4-day Aldara (50 mg)-treated mice were (a) probed for TUNEL positivity (Cell Death Detection Kit [Roche, Basel, Switzerland]); and (b) analyzed for epidermal TUNEL positive cells/field. (c) Immunoblots of skin homogenates were probed for indicated proteins. 4-day Aldara-treated skin explants were cultured (DMEM, 24 hours) before analysis for (d) LDH, (e) IL-1β, and (f) IL-18 release. Control (n = 2) and Aldara (n = 5). Healthy untreated back skin sections from Casp-11+/+ and Casp-11−/−.
The IL-23–IL-17 axis is the main driver of pathogenesis in psoriasis, as evidenced by successful therapies that target these cytokines (Lowes et al., 2014). In murine skin, IL-1β stimulates keratinocytes to produce chemotaxants for immune cells, proliferation of γδ T cells, and production of IL-17 (Cai et al., 2019; Ghoreschi et al., 2010). IL-1β mRNA and protein levels in patient psoriatic skin correlate with disease progression and treatment response (Cai et al., 2019), and blocking IL-1β is effective in patients with pustular psoriasis and psoriatic arthritis (Tsai and Tsai, 2017). The involvement of IL-1β in cutaneous inflammation implies a central role for inflammatory caspases in the pathogenesis of psoriasis. Expression and activation of inflammatory caspases is upregulated in psoriatic lesions (Johansen et al., 2007; Salskov-Iversen et al., 2011; Zwicker et al., 2017). Imiquimod (IMQ), the active component of Aldara cream (5% IMQ), is a TLR7/8 agonist that induces the development of an inflammatory skin disease remarkably similar to psoriasis (van der Fits et al., 2009). Significant amelioration of IMQ-induced murine skin inflammation was recently demonstrated by the genetic deficiency or pharmacological inhibition of both caspase-1 and caspase-11 (Aira et al., 2019). However, caspase-1 and caspase-11 may have nonredundant roles during psoriasis, and their individual contribution to its pathogenesis remains to be addressed. There are conflicting reports regarding the function of caspase-1 during psoriasis (Cho et al., 2012; Rabeony et al., 2015); thus, we aimed to determine the specific role of caspase-11 during IMQ-induced skin inflammation. All experiments were performed under license and approval of the Trinity College Dublin animal research ethics committee and the Irish Health Protection Regulatory Agency.

Following application of Aldara to the back skin of Casp-11+/+ and Casp-11−/− mice for four consecutive days, there was significantly less scaling and erythema in Casp-11−/− skin than Casp-11+/+ (Figure 1a–c). Less epidermal thickening in Casp-11−/− skin was also observed at early stages of disease (Supplementary Figure S1 online). Psoriatic lesions typically display increased acanthosis, immune cell infiltration, and angiogenesis. Histologic examination of IMQ-treated skin demonstrates that Casp-11−/− skin displays significantly less epidermal proliferation (Figure 1d and e) and leukocyte infiltration into the dermis (Figure 1f and g). The reduction in erythema seen in Casp-11−/− skin correlates with significantly fewer endothelial cells in the dermis (Figure 1h and i), decreased mRNA expression of the proangiogenic marker Ang-2 (Figure 1j), and increased expression of the antiangiogenic marker TSP-1 (Figure 1k). These results reveal that caspase-11 contributes to IMQ-induced skin pathology by promoting keratinocyte proliferation, immune cell infiltration, and neoangiogenesis to support the metabolic demands of the uncontrolled keratinocytes.

Analysis of the epidermal layer revealed that IMQ-treated Casp-11−/− skin had significantly less TUNEL positivity than Casp-11+/+ skin (Figure 2a and b). TUNEL staining identifies both apoptotic and pyroptotic cells; thus, both pathways were further examined. Although caspase-11 has been reported to promote caspase-3 processing after lipopolysaccharide challenge (Kang et al., 2002), no differences in caspase-3 processing were observed (Figure 2c). Caspase-11 mediates pyroptosis via Gasdermin-D cleavage, resulting in pore formation and release of inflammatory mediators such as IL-1β and lactate dehydrogenase. We show that Gasdermin-D cleavage, and consequent lactate dehydrogenase release, are significantly lower in Casp-11−/− skin than Casp-11+/+ (Figure 2c and d), confirming a caspase-11 requirement for pyroptotic cell death during this disease. Decreased cell death in both the dermis and epidermis of Casp-11−/− skin was observed as early as 24 hours following Aldara treatment (Supplementary Figure S2a and b online). The absence of caspase-11 did not alter the secretion of inflammasome-dependent cytokines from IMQ-treated skin (Figure 2e and f). Aira et al. (2019) recently reported decreased IL-1β and IL-18 secretion from caspase-1/caspase-11 double knockouts, suggesting that caspase-1, rather than caspase-11, is required for the secretion of inflammasome-mediated cytokines from skin. However, when healthy Casp-11−/− and Casp-11+/+ skin were stimulated with Aldara ex vivo, and conditioned media from Aldara-treated skin was subsequently applied to bone marrow–derived macrophages, less inflammasome-mediated cytokine secretion occurred in Casp-11−/− bone marrow–derived macrophages (Figure 2g and h). Findings suggest that caspase-11-mediated pyroptosis in the skin induces the secretion of alarmins that serve to drive inflammasome activation in immune cells. Caspase-11 also contributes to IL-1β secretion in response to conditioned media from Aldara-treated skin (Figure 2g and h). The role of pyroptosis has not been studied in detail in psoriasis pathology; however, our results suggest that inhibiting inflammasome activation could be beneficial for patients with psoriasis. Dataset analysis reveals that inflammatory caspase-4 and -5, pro–IL-1β, and the pyroptotic mediator Gasdermin-D are all significantly elevated in human psoriatic lesions (Supplementary Figure S3 online). Alarmins (including heat shock proteins, mitochondrial components, and S100 proteins) are enriched in secretomes from human psoriatic lesions (Williamson et al., 2013), suggesting that proinflammatory cell death is also occurring in human lesions. Treatment with NLRP3 (Irrera et al., 2017) or inflammatory caspase (Aira et al., 2019) inhibitors has already been shown to ameliorate IMQ-induced murine psoriasis. This study identifies a clear and singular role for caspase-11 in mediating IMQ-induced skin inflammation and proposes further
investigation of caspase-4/5 inhibition for the treatment of inflammatory skin conditions.

Data availability statement
All data generated or analyzed during this study are included in this published article (and its supplementary information files).

ORCIDs
Sinead Kenealy: https://orcid.org/0000-0003-4666-146X
Joan Manils: https://orcid.org/0000-0001-8429-1295
Mathilde Raverdoue: https://orcid.org/0000-0002-6088-6902
Natalia Munoz-Wolf: https://orcid.org/0000-0002-5173-4026
Gillian Barber: https://orcid.org/0000-0002-6909-6042
Alex Liddicoat: https://orcid.org/0000-0002-1841-2033
Ed C. Lavelle: https://orcid.org/0000-0002-3167-1080
Emma M. Creagh: https://orcid.org/0000-0001-7631-4370

CONFLICT OF INTEREST
The authors state no conflict of interest.

ACKNOWLEDGMENTS
We thank the J. Yuan laboratory (Harvard Medical School) for gifting Casp-11** mice. This study was supported by an SFI Investigator program grant (12/IP/1400).

AUTHOR CONTRIBUTIONS
Conceptualization: SK, JM, EMC; Data Curation: SK, JM, MR, NMW, GB, AL; Funding Acquisition: EMC; Resources: ECL; Supervision: EMC; Writing - Original Draft Preparation: SK, JM, EMC; Writing - Review and Editing: SK, JM, EMC, ECL.

TO THE EDITOR
The acute itch-scratch reflex, considered a protective and evolutionarily conserved mechanism, can become dysfunctional in the setting of many chronic skin diseases, resulting in chronic itch (Mollanazar et al., 2016; Steinhoff et al., 2018; Talwalkar et al., 2003). The resultant debilitating itch-scratch cycle can occur in inflammatory skin diseases; neurological diseases (Meng et al., 2018; Steinhoff et al., 2012); and systemic conditions such as cancer, diabetes, and renal or hepatic disorders.

REFERENCES
Cho KA, Suh JW, Lee KH, Kang JL, Woo SY. IL-17 and IL-22 enhance skin inflammation by stimulating the secretion of IL-1beta by keratinocytes via the ROS-NLRP3-caspase-1 pathway. Int Immunol 2012;24:147−58.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.
Supplementary Figure S1. Histological H&E staining of skin sections show that less epidermal thickness occurs between Casp-11+/+ and Casp-11−/− mice during IMQ-induced psoriasis. Mice were treated with Aldara (50 mg) for 24 hours. Back whole skin tissue was fixed in formalin and paraffin embedded. (a) Representative images of H&E-stained control and Aldara-treated back skin taken at 24 hours. (b) Epidermal thickness was measured (average of three images per piece of tissue, and three pieces of tissue per mouse) in 24-hour treated skin. Thickness was measured in nm using Image J software. Control (n = 2) and Aldara (n = 5), error bars represent mean ± SEM. Two-way ANOVA followed by Bonferroni post-test found *P < 0.05. ANOVA, analysis of variance; H&E, hematoxylin and eosin; IMQ, imiquimod; SEM, standard error of the mean. 10× magnification of indicated regions, Bar = 20 μm.
Supplementary Figure S2. Less cell death is observed in FFPE skin of Casp-11−/− mice at early stages of experimental psoriasis. Casp-11+/+ and Casp-11−/− mice were treated with Aldara (50 mg) for 24 hours. Back whole skin tissue was fixed in formalin and paraffin embedded. (a) Representative images of TUNEL-stained control and Aldara-treated back skin taken at 24 hours. (b) TUNEL positive cells were counted/field in the epidermis in an average of three images per piece of tissue, and three pieces of tissue per mouse. Control (n = 2) and Aldara (n = 5), error bars represent mean ± SEM. Two-way ANOVA followed by Bonferroni post-test found *P < 0.05. ANOVA, analysis of variance; SEM, standard error of the mean; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling. 10× magnification, Bar = 20 μm.
Supplementary Figure S3. Enhanced expression of pyroptosis-related genes in biopsy tissue from human psoriatic lesions. Relative mRNA expression levels of (a) caspase-4, (b) caspase-5, (c) IL-1β, and (d) Gasdermin-D in normal, uninvolved, and lesioned human psoriatic skin were analyzed using the published dataset, GDS4602 (Nair et al., 2009). For each indicated gene, graphs indicate the gene expression values in individual samples. One-way ANOVA test was used to evaluate the significant differences among the three groups. *P < 0.05, ****P < 0.0001. ANOVA, analysis of variance.