

Accepted Manuscript

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PII: S0022-202X(19)31624-0

DOI: <https://doi.org/10.1016/j.jid.2019.05.010>

Reference: JID 1918

To appear in: *The Journal of Investigative Dermatology*

Received Date: 17 April 2019

Revised Date: 10 May 2019

Accepted Date: 19 May 2019

Please cite this article as: Kenealy S, Manils J, Raverdeau M, Munoz-Wolf N, Barber G, Liddicoat A, Lavelle EC, Creagh EM, Caspase-11 Mediated Cell Death Contributes to the Pathogenesis of Imiquimod-Induced Psoriasis, *The Journal of Investigative Dermatology* (2019), doi: <https://doi.org/10.1016/j.jid.2019.05.010>.

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CASPASE-11 MEDIATED CELL DEATH CONTRIBUTES TO THE PATHOGENESIS OF IMIQUIMOD-INDUCED PSORIASIS

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Abbreviations used: IMQ, Imiquimod; casp1/11, caspase-1 and -11; DKO, double knock-out; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling; LPS, lipopolysaccharide; LDH, lactate dehydrogenase; BMDM, bone marrow derived macrophage; HSP, heat shock protein.

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-1 β 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 autoinflammatory events, governed by IL-1 β (Martinez-Quiles and Goldbach-Mansky, 2018), prevail in pustular forms of psoriasis (Liang et al., 2017).

The IL-23-IL-17 axis is the main driver of pathogenesis in psoriasis, as evidenced by successful therapies which target these cytokines (Lowes et al., 2014). In murine skin, IL-1 β stimulates keratinocytes to produce chemoattractants for immune cells, proliferation of $\gamma\delta$ T cells, and production of IL-17 (Cai et al., 2019) (Ghoreschi et al., 2010). IL-1 β mRNA and protein levels in patient psoriatic skin correlate with disease progression and treatment response (Cai et al., 2019), and blocking IL-1 β blockers is effective in pustular psoriasis and psoriatic arthritis patients (Tsai and Tsai, 2017). The involvement of IL-1 β 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 -11 (casp1/11) (Aira et al., 2018). However casp1/11 may have non-redundant roles during psoriasis, and their individual contribution to its pathogenesis remain 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 TCD animal research ethics committee and the Irish Health Protection Regulatory Agency (HPRA).

Following application of Aldara to the back skin of *Casp-11^{+/+}* and *Casp-11^{-/-}* mice for 4 consecutive days, there was significantly less scaling and erythema in *Casp-11^{-/-}* skin, as compared to *Casp-11^{+/+}* (Figure 1a-c). Less epidermal thickening in *Casp-11^{-/-}* skin was also observed at early stages of disease (Figure S1). Psoriatic lesions typically display increased acanthosis, immune cell infiltration and angiogenesis. Histological examination of Aldara-treated skin demonstrates that *Casp-11^{-/-}* skin displays significantly less epidermal proliferation (Figure 1d, e) and leukocyte infiltration into the dermis (Figure 1f, g). The reduction in erythema seen in *Casp-11^{-/-}* skin correlates with significantly less endothelial cells in the dermis (Figure 1h, i), decreased mRNA expression of the pro-angiogenic marker, Ang-2 (Figure 1j), and increased expression of the anti-angiogenic maker, 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 Aldara-treated *Casp-11^{-/-}* skin had significantly less TUNEL positivity than *Casp-11^{+/+}* skin (Figure 2a, 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 LPS 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-1 β and LDH. We show that Gasdermin-D cleavage, and consequent LDH release, are significantly lower in *Casp-11*^{-/-} skin compared to *Casp-11*^{+/+} (Figure 2c, 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 h following Aldara treatment (Figure S2a, b). The absence of caspase-11 did not alter the secretion of inflammasome-dependent cytokines from Aldara-treated skin (Figure 2e, f). Aria *et al.* recently reported decreased IL-1 β and IL-18 secretion from casp-1/11 DKO (Aira et al., 2018), 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 BMDM, less inflammasome-mediated cytokine secretion occurred in *Casp-11*^{+/+} BMDM (Figure 2g, 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-1 β secretion in response to conditioned media from Aldara-treated skin (Figure 2g, 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 psoriatic patients. Dataset analysis reveals that inflammatory caspases-4 and -5, pro-IL-1 β , and the pyroptotic mediator, Gasdermin D, are all significantly elevated in human psoriatic lesions (Figure S3). Alarmins (including HSPs, mitochondrial components and S100 proteins) are enriched in secretomes from human psoriatic lesions (Williamson et al., 2013), suggesting that pro-inflammatory cell death is also occurring in human lesions. Treatment with NLRP3 (Irrera et al., 2017) or inflammatory caspase (Aira et al., 2018) inhibitors has already been shown to

ameliorate Aldara-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 analysed during this study are included in this published article (and its supplementary information files).

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgements

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

CRediT statement

SK and JM contributed to the study conceptualization and generated the majority of the data presented, assisted by MR, NMW, GB and AL. ECL contributed to resources and editing. EMC provided funding, conceptualization and supervision of the study. SK, JM and EMC wrote and edited the manuscript.

References:

- Aira LE, Goncalves D, Bossowski JP, Rubio-Patino C, Chiche J, Paul-Bellon R, et al. Caspase 1/11 deficiency or pharmacological inhibition mitigates psoriasis-like phenotype in mice. *The Journal of investigative dermatology* 2018.
- Cai Y, Xue F, Quan C, Qu M, Liu N, Zhang Y, et al. A Critical Role of the IL-1 β -IL-1R Signaling Pathway in Skin Inflammation and Psoriasis Pathogenesis. *The Journal of investigative dermatology* 2019;139(1):146-56.
- Cho KA, Suh JW, Lee KH, Kang JL, Woo SY. IL-17 and IL-22 enhance skin inflammation by stimulating the secretion of IL-1 β by keratinocytes via the ROS-NLRP3-caspase-1 pathway. *International immunology* 2012;24(3):147-58.
- Creagh EM. Caspase crosstalk: integration of apoptotic and innate immune signalling pathways. *Trends in immunology* 2014;35(12):631-40.

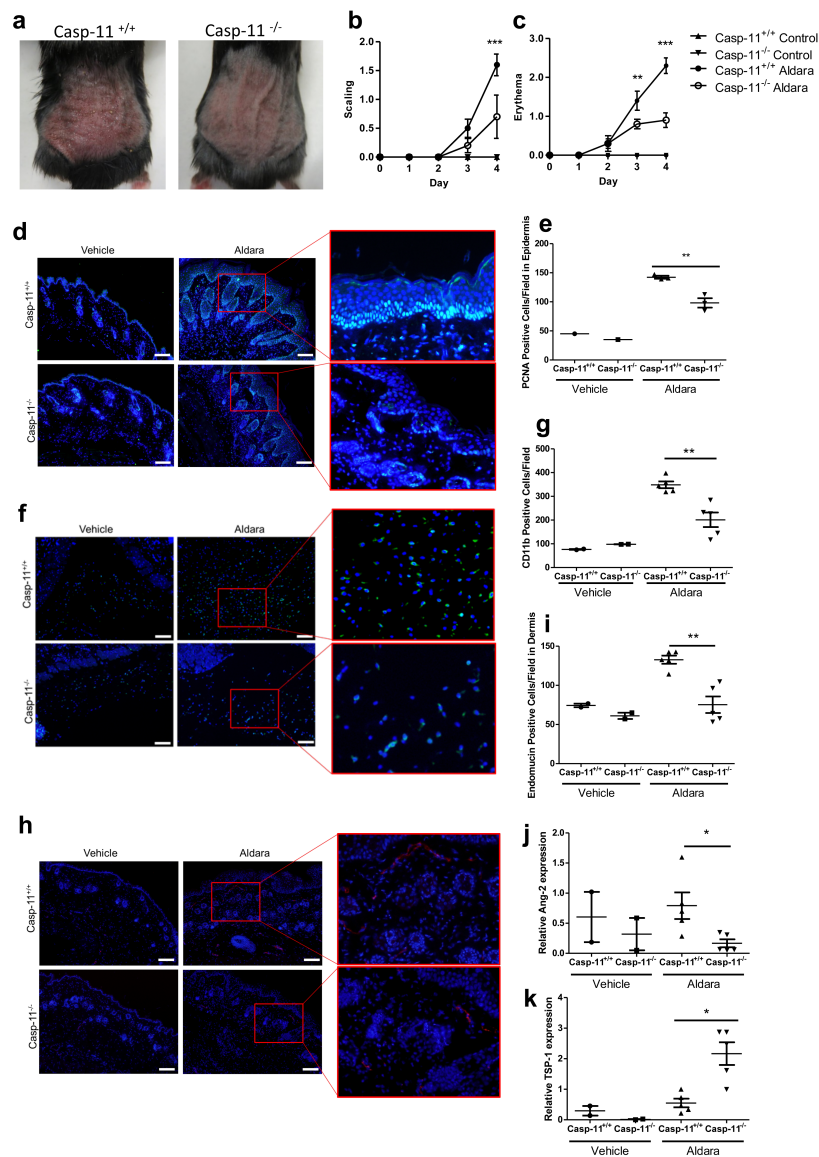
- Ghoreschi K, Laurence A, Yang XP, Tato CM, McGeachy MJ, Konkel JE, et al. Generation of pathogenic T(H)17 cells in the absence of TGF-beta signalling. *Nature* 2010;467(7318):967-71.
- Irrera N, Vaccaro M, Bitto A, Pallio G, Pizzino G, Lentini M, et al. BAY 11-7082 inhibits the NF-kappaB and NLRP3 inflammasome pathways and protects against IMQ-induced psoriasis. *Clinical science (London, England : 1979)* 2017;131(6):487-98.
- Johansen C, Moeller K, Kragballe K, Iversen L. The activity of caspase-1 is increased in lesional psoriatic epidermis. *The Journal of investigative dermatology* 2007;127(12):2857-64.
- Kang SJ, Wang S, Kuida K, Yuan J. Distinct downstream pathways of caspase-11 in regulating apoptosis and cytokine maturation during septic shock response. *Cell death and differentiation* 2002;9(10):1115-25.
- Liang Y, Sarkar MK, Tsoi LC, Gudjonsson JE. Psoriasis: a mixed autoimmune and autoinflammatory disease. *Current opinion in immunology* 2017;49:1-8.
- Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of psoriasis. *Annual review of immunology* 2014;32:227-55.
- Martinez-Quiles N, Goldbach-Mansky R. Updates on autoinflammatory diseases. *Current opinion in immunology* 2018;55:97-105.
- Rabeony H, Pohin M, Vasseur P, Petit-Paris I, Jegou JF, Favot L, et al. IMQ-induced skin inflammation in mice is dependent on IL-1R1 and MyD88 signaling but independent of the NLRP3 inflammasome. *European journal of immunology* 2015;45(10):2847-57.
- Salskov-Iversen ML, Johansen C, Kragballe K, Iversen L. Caspase-5 expression is upregulated in lesional psoriatic skin. *The Journal of investigative dermatology* 2011;131(3):670-6.
- Tsai YC, Tsai TF. Anti-interleukin and interleukin therapies for psoriasis: current evidence and clinical usefulness. *Therapeutic advances in musculoskeletal disease* 2017;9(11):277-94.
- van der Fits L, Mourits S, Voerman JS, Kant M, Boon L, Laman JD, et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J Immunol.* 2009;182(9):5836-45.
- Williamson JC, Scheipers P, Schwämmle V, Zibert JR, Beck HC, Jensen ON. A proteomics approach to the identification of biomarkers for psoriasis utilising keratome biopsy. *J Proteomics.* 2013;94:176-85.
- Zwicker S, Hattinger E, Bureik D, Batycka-Baran A, Schmidt A, Gerber PA, et al. Th17 micro-milieu regulates NLRP1-dependent caspase-5 activity in skin autoinflammation. *PloS one* 2017;12(4):e0175153.

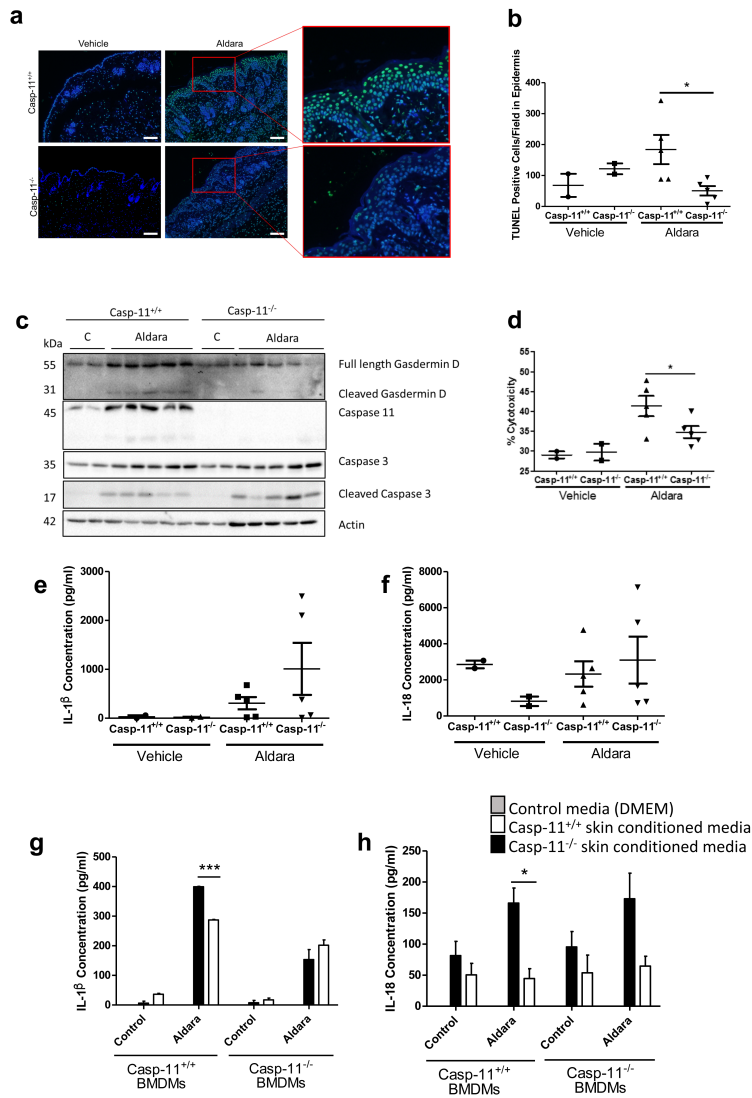
Figure legends

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 d, 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 +ve cells/field). Skin sections were probed and counted for (f, g) CD11b and (h, i) endomucin (10X

magnification, scale bars = 20 μ m). 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 $\pm$ SEM. Data is representative of three independent experiments. Two-way ANOVA, followed by Bonferroni post-test, was used to analyse statistical significance between *Casp-11*^{+/+} and *Casp-11*^{-/-} groups *p<0.05, ** p < 0.01, *** p < 0.001.

Figure 2: Caspase-11 mediated pyroptosis contributes to the inflammatory phenotype of IMQ-induced psoriasis. Skin sections from 4 d Aldara (50 mg) treated mice were (a) probed for TUNEL positivity (Cell Death Detection Kit (Roche)) (10X magnification, scale bars = 20 μ m); and (b) analysed for epidermal TUNEL +ve cells/field. (c) Immunoblots of skin homogenates were probed for indicated proteins. 4 d Aldara treated skin explants were cultured (DMEM, 24 h) prior to analysis for (d) LDH, (e) IL-1 β and (f) IL-18 release. Control (n=2) and Aldara (n=5). Healthy untreated back skin sections from *Casp-11*^{+/+} and *Casp-11*^{-/-} mice (n=5/group) were cut into three equal pieces and cultured for 24 h in the absence/presence of Aldara (17mg in 1.5ml DMEM). Sterile filtered supernatants (conditioned media) were cultured (50:50 ratio with DMEM) with BMDM for 24 h before determining (g) IL-1 β and (h) IL-18 secretion. Error bars represent mean $\pm$ SEM. Data is representative of three independent experiments. Two-way ANOVA, followed by Bonferroni post-test, was used to analyse statistical significance between *Casp-11*^{+/+} and *Casp-11*^{-/-} groups *p<0.05, *** p < 0.001.





Supplementary Figure Legends

Kenealy S. *et al.* 'Caspase-11 mediated cell death contributes to the pathogenesis of Imiquimod-induced psoriasis'

Figure S1: Histological H&E staining of skin sections show that less epidermal thickness occurs between *Casp-11*^{+/+} and *Casp-11*^{-/-} mice during Imiquimod-induced psoriasis.

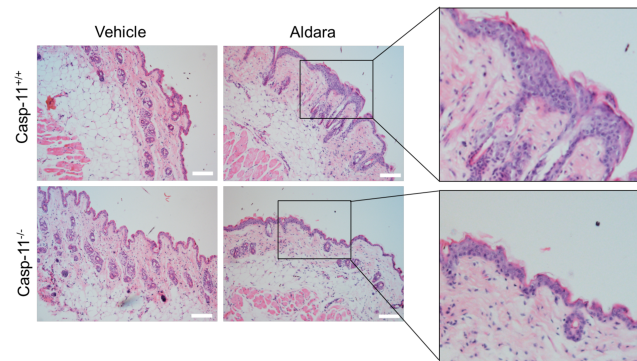
Mice were treated with Aldara (50 mg) for 24 h. Back whole skin tissue was fixed in Formalin and paraffin embedded. (a) Representative images of Haematoxylin and Eosin stained control and Aldara treated back skin taken at 24 h. Scale bar = 20 μ m; 10X magnification of indicated regions. (b) Epidermal thickness was measured (average of three images per piece of tissue, and three pieces of tissue per mouse) in 24 h treated skin. Thickness was measured in nm using Image J software. Control (n=2) and Aldara (n=5), error bars represent mean $\pm$ SEM, Two-way Anova followed by Bonferroni post-test found * $p < 0.05$.

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 h. Back whole skin tissue was fixed in formalin and paraffin embedded (FFPE). (a) Representative images of TUNEL stained control and Aldara treated back skin taken at 24 h. (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. Scale bar = 20 μ m; 10X magnification. Control (n=2) and Aldara (n=5), error bars represent mean $\pm$ SEM, Two-way Anova followed by Bonferroni post-test found * $p < 0.05$.

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-1 β ; and (d) Gasdermin D; in normal, uninvolved and lesioned human psoriatic skin were analysed 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 between the 3 groups. * $p < 0.05$, **** $p < 0.0001$.

a**b**