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TRPV1 mediates inflammation and hyperplasia in imiquimod (IMQ)-induced psoriasiform dermatitis (PsD) in mice

Running title: TRPV1 enhances the IMQ-induced PsD of mice

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Highlights

● Imiquimod-induced psoriasiform dermatitis in TRPV1 KO mice is markedly diminished.

● TRPV1 plays a role in epidermal barrier dysfunction of psoriasiform dermatitis.

● TRPV1 regulates accumulation of inflammatory cells in psoriasiform dermatitis.

● Inflammatory cytokine genes are mediated by TRPV1 in psoriasiform dermatitis.

● TRPV1 is a potential target for treatment in psoriasiform dermatitis.

Abstract

Background: Transient Receptor Potential Vanilloid 1 (TRPV1) is known to mediate itch and neurogenic inflammation, but the role of TRPV1 in psoriasiform dermal inflammation is poorly understood.

Objective: To investigate the function of TRPV1 in imiquimod (IMQ)-induced psoriasiform dermatitis (PsD) in mice.

Methods: Following daily treatment of topical IMQ cream for consecutive 5 days in C57BL/6 wide-type (WT) and TRPV1 gene knockout (KO) mice, we assessed the psoriasis severity index (PSI) scores, transepidermal water loss (TEWL), dermal inflammatory infiltrates, as

well as gene expression levels for psoriasis related genes in mouse skin lesions.

Results: Compared with WT mice, the clinical and TEWL scores, the extent of skin

hyperplasia, the area of Munro microabscesses (MM) and angiogenesis of psoriasis were all

significantly decreased in TRPV1 KO mice triggered with IMQ, suggesting a reduction in

skin inflammation and barrier defects. In addition, the infiltration of CD45+ leukocytes, mast

cells as well as CD3+ T cells was all reduced in the IMQ-treated skin of TRPV1 KO mice.

Quantitative Real-time PCR (RT-qPCR) revealed that expression levels of IL-1β, IL-6, IL-23,

S100A8 were decreased while IL-10 was increased in TRPV1 KO mice.

Conclusions: In summary, key markers of psoriatic inflammation and epidermal hyperplasia

are reduced in TRPV1 KO mice, indicating the involvement of TRPV1 in the psoriasiform

inflammation and suggesting its potential as a therapeutic target.

1. Introduction

Psoriasis is a chronic cutaneous inflammatory condition, which is mediated by dermal

leukocytes, including dendritic cells (DCs), T cells, mast cells and neutrophils[1]. T cell

activation is thought to be initiated and driven by DCs which produce various pro-

inflammatory cytokines, including IL-6 and IL-23, further driving Th17 cell-mediated

inflammation[2]. The following histological features of psoriasis are generally accepted: a

greatly thickened epidermis with elongated rete ridges into the dermis, an increased number

of prominent blood vessels in the papillary dermis, and the presence of a collection of

neutrophils in the cornified layer known as a Munro's microabscess (MM) [3]. Interestingly,

there are clinical reports that psoriasis is worsened by psychosocial stress[4]. Nerve

damage/denervation had resulted in localized clearance of psoriasis in the distribution of the

nerve and that the psoriatic lesions recurred with recovery of cutaneous sensation[5]. TRP

channels, such as Transient Receptor Potential Vanilloid 1 (TRPV1), are involved in

neuropeptide release [6] and have been implicated in susceptibility to inflammation of

psoriasiform dermatitis (PsD) [7]. TRPV1, a Ca2+ permeable channel, is involved in the

pathogenesis of both inflammatory and neuropathic pain and itch. Pruritus induced by IL-31,

a key inflammatory marker in atopic dermatitis (AD), is also mediated by TRPV1[8]. TRPV1

antagonists may expand the dermatologist's armamentarium against AD[9], but its role in

other skin inflammatory disorders, such as psoriasis, is poorly understood.

TRPV1 played a pro-inflammatory role in some neurological disorders by amplifying the

effects of IL-1β and IL-6 [10]. Immune cells in the skin, such as mononuclear cells, DCs and

mast cells, not only express TRPV1, but also are directly affected by TRPV1 activation[11].

Roblin *et al.* reported that CT327 (a topical TrkA kinase inhibitor) inhibited nerve growth

factor (NGF)-TrkA-TRPV1 pathway, thereby reducing pruritus in psoriatic patients[12].

Leigh *et al.* reported that overexpression of TRPV1 in lesions of patients was positively

correlated to itch of psoriasis[13]. Experimentally, chemical inhibition of TRPV1+NaV1.8+

nociceptors by resiniferatoxin (RTX) reduced IL-23/IL-17 responses in imiquimod (IMQ)-

induced psoriasiform inflammation[7]. RTX, however blocks TRPV1 as well as TRPA1 and

thus is not specific for TRPV1[14]. It has never been clearly addressed how TRPV1 affects

the immune cells in terms of cellular types, infiltration patterns, accumulation numbers, and

related skin environment changes. Therefore, our specific aims were to investigate the

function of TRPV1 in skin inflammation by quantitatively and qualitatively comparing skin

alterations in TRPV1 gene knockout (KO) vs. wild-type (WT) mice. Our results indeed

suggest that TRPV1 mediates inflammation in the IMQ-based psoriasis model.

2. Materials and Methods

2.1. Animals

Animal experiments were approved by the University of California, Davis, Animal Care

and Use Committee. Female and male c57BL/6 WT and TRPV1 KO mice (purchased from

JACKSON Laboratory), (8-10 weeks, 20-25g) were used.

2.2. Induction of psoriasiform skin inflammation

The fur on the rostral back was shaved >5 days prior to treatment. PsD was induced by

topically applying 50mg of 5% IMQ cream (Aldara, 3M Health Care Limited, UK), or control

vehicle cream (Vanicream; Pharmaceutical Specialties, Rochester, MN) once daily for 5

consecutive days. Mice were euthanized on day 5. The treated skin was collected and then

stored in 3 parts immersed in RNA later, 10% paraffin and optimal cutting temperature (OTC)

freezing medium and stored in -80℃.

2.3. Transepidermal water loss (TEWL)

TEWL [15] was measured by applying a Vapo Meter® (Delfin Technologies Ltd., Kuopio,

Finland) to the skin for 10s.

2.4. Skin Score

Assessment of local modified psoriasis severity index (PSI) of PsD inflammation: the PSI

score assessment is used to monitor the progress of the psoriatic inflammation in mice on day

5. The PSI score includes PSI-E (erythema), PSI-S (scaling) and PSI-I (induration). PSI-

E/PSI-S/PSI-I was evaluated and graded 0 to 4 (0: absent to 4: very severe)[16] by a blinded

observer. Total PSI score is 0 to 12.

2.5. Histology and immunohistochemistry (IHC)

Skin tissue samples were formalin-fixed (10%) and embedded in paraffin for Hematoxylin

and Eosin (H&E) or for Giemsa staining (Mast cells). We assessed histopathological

alterations (epidermal thickness and area of MM) with computer-assisted quantitative image

analysis (Image J) in lesions. Tissue sections (5μm) were prepared for rabbit polyclonal anti-

Ki67(diluted in 1:1000, Clone#Ab-4, NeoMarkers, U.S.A.), rabbit monoclonal anti-

pSTAT3(diluted in 1:200,Clone#G.374.10,Thermo Scientific, U.S.A.), goat polyclonal anti-

CD31(diluted in 1:1800, Clone#M-20, Santa Cruz, U.S.A.), rat polyclonal anti-CD45(diluted

in 1:400,Clone#OX-1, BD Pharmingen, U.S.A.) and rabbit monoclonal anti-CD3(diluted in

1:800, Clone#Sp7, Abcam, U.S.A.) for IHC. Individual inflammatory cell types CD45 were

counted in 5 microscopic fields at 400X and expressed as cells/HPF, with mean±S.D. Ki67,

pSTAT3 and CD3 positive cells and mast cells were counted in 5 microscopic fields at 200X

and expressed as cells/Field (200X), with mean±S.D.

2.6. Quantitative Real-time PCR (RT-qPCR)

RNA was extracted from cryopreserved skin specimens with Mini Spin Columns to

perform gene expression analyses using RT-qPCR with SYBR Green methods. Primers for

IL-23 (Mm.PT.58.10594618.g), IL-6 (Mm.PT.58.10005566), IL-1β (Mm.PT.58.10005566), S100A8 (Mm.PT.58.44003402.gs), CXCL1 (Mm.PT.58.42076891), IL-10 (Mm.PT.58.-13531087)，IL-17A (Mm.PT.58.6531092), IL-17F (Mm.PT.58.9739903)，IL-22(L: 5'-CTG CTT CTC ATT GCC CTG TG-3'; R: 5'-AGC ATA AAG GTG CGG TTG AC-3') and DEFB4 (Mm.PT.58.29993789) were designed by Integrated DNA Technologies, Inc., Iowa, USA.

2.7. Statistical analysis

All results were expressed as mean ± S.D. and analyzed statistically with Graphpad Prism 6.02（Graphpad Software, Inc., San Diego, USA）. The student t test was used to analyze the differences between two groups. One-way ANOVA followed post-hoc comparison (Tukey's HSD) test were used to compare the differences in more than two groups. Statistical significance was defined as $P<0.05$.

3. Results

3.1. IMQ-induced psoriasiform dermatitis is alleviated in TRPV1 KO mice

We compared the clinical and immunologic differences in the skin of TRPV1 KO mice compared to WT control littermates using a well-established model of PsD. After topically applying IMQ for consecutive 5 days, we noticed that the clinical manifestations of PsD were less severe in the TRPV1 KO group, compared with WT group (Fig. 1A). To confirm this observation, PSI scores were assigned to mice by a blinded observer. Compared with WT mice, the total PSI scores of TRPV1 KO mice ($P<0.05$) were decreased, which was shown for both the scores of scaling (PSI-S) ($P<0.01$) and induration (PSI-I) ($P<0.05$) (Fig.1B).

Disruption of the skin barrier function can be measured objectively through a device that

quantifies TEWL. An increase in TEWL, for example, would reflect a loss of barrier function and is accompanied by perceived skin dryness and visible surface scales. TEWL has been reported to increase in psoriasis patients and to go down following successful therapy [17]. As might be expected, TEWL increased significantly in WT mice treated with IMQ upon development of psorisiform changes. TEWL, however, in TRPV1 KO mice was decreased by 49.55% (P<0.0001) after topical application of IMQ for 5 days, as compared with WT mice (Fig.1C), suggesting better preservation of skin barrier function in the KO mice. Thus, TRPV1 contributes to the disrupted skin barrier damage following IMQ application, thus potentially explaining the reduction in scaling in psoriatic lesions of IMQ-treated TRPV1 KO mice.

3.2. Reduced epidermal thickness and proliferation in TRPV1 KO mice (compared to WT mice) following application of IMQ

Epidermal hyperproliferation and thickening is a hallmark of psoriatic inflammation. Histopathological examination showed prominent hyperkeratosis and acanthosis after treatment by IMQ on the back of mice for 5 days (Fig.2A). We measured epidermal thickness with Image J analysis from H&E skin sections. Epidermal hyperplasia was reduced in TRPV1 KO mice by 41.03% (P<0.0001) compared with WT mice (Fig.2B). Weakly positive staining of Ki67 (Fig.2C) and pSTAT3 (Fig.2D) were observed, respectively, in basal keratinocytes and dermis in both TRPV1 KO and WT mice treated with vehicle. In IMQ-treated groups, however, the numbers of Ki67+ and pSTAT3+ cells per microscopic field in WT mice were significantly greater than that in TRPV1 KO mice (Fig.2C,2D) (P<0.0001).

3.3. Angiogenesis and MM are decreased in TRPV1 KO mice compared to WT mice

Next, we determined if angiogenesis and the presence of MM, two prominent features of human psoriasis, were altered in TRPV1 KO mice. Evaluation of microvascular density (MVD) was done by immunohistochemical staining for CD31 (Fig. 3A). The average MVD in psoriatic lesions in WT group increased markedly (P<0.0001) with IMQ treatment compared with vehicle. Although TRPV1 KO mice seemed to have more angiogenesis in IMQ-treated mice than those treated with vehicle (P<0.0001), the extent of augment of MVD in TRPV1 KO mice were reduced by 44.28% (P<0.0001) compared to WT mice (Fig.3B). Thus, the profound angiogenesis in the skin of IMQ-treated mice appears to be partially dependent on TRPV1.

Finally, we measured MM formation in lesional psoriatic lesion of WT and TRPV1 KO mice and found that MM were diminished in the latter group (Fig. 3C). The average area of total MM per mouse was reduced in TRPV1 KO mice by 57.61% (P<0.05) compared with WT mice (Fig. 3D), demonstrating that TRPV1 plays a role in neutrophils accumulation in the IMQ-treated skin.

3.4. Reduction in infiltration of inflammatory cells in IMQ-treated skin of TRPV1 KO mice

In order to assess the clinical and pathological influence of TRPV1 on the composition of dermal inflammatory cells following IMQ treatment, we used IHC or Giemsa staining (for mast cells) in WT and TRPV1 KO mice following induction of PsD. We observed large numbers of CD45+ leukocytes (Fig. 4A), scattered staining of CD3+ T cells (Fig. 4B) and mast cells (Fig. 4C) in the IMQ-treated lesional skin. The average numbers of CD45+, mast

cells and CD3+ cells per microscopic field showed significant increases in WT mice following IMQ treatment compared to vehicle treatment. Interestingly, the numbers in IMQ-treated WT mice were significantly higher (P<0.0001) than that in IMQ-treated TRPV1 KO mice (Fig. 4A, 4B, 4C). These data suggest that dermal inflammatory cell infiltration, which may include proliferation, was inhibited with the absence of TRPV1 in the psoriasis model.

3.5. mRNA expression of related genes in psoriasis lesions

To understand the mechanism whereby TRPV1 mediates psoriasiform inflammation, we measured mRNA expression for key inflammation genes that are known to regulate psoriatic skin inflammation. RT-qPCR showed relatively decreased mRNA levels of IL-1β (P<0.001), IL-6(P<0.05), IL-23(P<0.05), S100A8(P<0.05), CXCL1(P=0.06) and increased level of IL-10 (P<0.01) in the psoriasiform skin lesions of TRPV1 KO mice as compared with WT mice. The mRNA levels of IL-17A, IL-17F, IL-22 and DEFB4 were somewhat lower in TRPV1 KO (vs. WT) mice although this did not reach statistical significance (P>0.05) (Fig. 5). Thus, TRPV1 impacts skin inflammatory changes by regulating important pro-inflammatory cytokine genes such as IL-1β and IL-6, but not necessarily IL-17A/F, in this experimental model of psoriasis.

4. Discussion

There has been interest in the role of TRP channel receptors in inflammatory skin diseases such as psoriasis. Von Andrian *et al* reported that resiniferatoxin (RTX) reduced IL-23/IL-17 responses in IMQ-induced psoriasiform inflammation. They concluded that NaV1.8+TRPV1+ nociceptors are driving to induce IL-23 production. Subsequently, IL-17A, IL-17F and IL-22

were reduced significantly in RTX-treated mice with ELISA[7]. Because TRPA1 and TRPV1

are co-expressed in sensory neurons, however, treatment with RTX on TRPV1+ neurons

reduces the expression of both TRPV1 and TRPA1[14], and RTX treatment decreases mRNA

expression of nociceptor markers, including not only TRPV1 and TRPA1 but also Substance P

and TRPM8 in dorsal root ganglia (DRGs) in IMQ induced PsD [7]. More recently, Ágnes

Kemény *et al*. reported that TRPV1 KO mice also showed fewer changes in skin thickness

and blood perfusion in comparison to WT mice following IMQ treatment [18], but their

treatment period was somewhat shorter than that reported by other investigators. Herein, we

used a variety of methodologies, including PSI scores, TEWL, dermal inflammatory

infiltrates, as well as gene expression levels to investigate the role of TRPV1 in IMQ-induced

psoriasiform dermatitis.

Psoriatic plaques appear characteristically dry and have fine silvery scales. To objectively

measure skin barrier function, we took advantage of technology that can measure TEWL.

Elevated TEWL scores in lesional skin of psoriasis patients has been reported to normalize

after use of emollients [19] and after effective light therapy[20]. We found that TEWL scores

increased markedly in mice treated with IMQ on day 5. This elevation was reduced by almost

a half in TRPV1 KO mice. Our data indicate that TRPV1 may contribute in the epidermal

barrier dysfunction that has been reported in psoriasis. Yun et al. reported that pharmacologic

blockade of TRPV1 activation can inhibit the symptoms of an AD-like condition as

manifested by a lower TEWL score [21]. The inhibition is based on restoration of a neutral

lipid layer and reversal of changes in loricrin (LOR) and filaggrin (FLG) expression[21]. FLG

and LOR, important epidermal barrier proteins, are decreased in psoriasis[22]. We speculate

that TRPV1's role in skin barrier damages may be related with above mechanisms or as consequence of inflammation.

We also found TRPV1 may participate in the recruitment of neutrophils, T cells and mast cells in skin as well as angiogenesis. A recent study has suggested that TRPV1 is expressed in CD4+ T cells where it contributes to T cell receptor-induced Ca2+ influx, signaling and T cell activation[23]. In addition, mast cells may be major producers of IL-22 in patients with psoriasis[24]. Our observations may be related to reduced mRNA levels of inflammatory cytokines, such as IL-1β, IL-6, IL-23, S100A8, and CXCL1 in the psoriatic lesion of TRPV1 KO mice induced by IMQ. The decrease of CXCL1 and other neutrophil chemoattractants in the TRPV1 KO mice may explain the marked diminished numbers of MM in the KO animals. It is notable that IL-10 mRNA is actually increased in the TRPV1 KO mice compared to WT littermates after IMQ treatment. IL-10 has been reported to have immunosuppressive functions. IMQ-treated IL-10 KO mice have been reported to show more severe psoriasiform skin features compared to WT controls [25], implying that IL-10 may have protective effect in psoriasis. Our data suggest that TRPV1 is a negative regulator of IL-10 expression.

Taken together, our study provides evidence that TRPV1 contributes to the development of psoriasiform dermatitis via its role in maximizing expression of several critical inflammatory cytokines such as IL-6, IL-23 and IL-1β. Interestingly, we did not detect a significant reduction IL-17A/F in TRPV1 KO mice, but this may be due to larger variation in the mRNA levels of these cytokines under the conditions tested. Our TEWL studies strongly suggest that epidermal barrier function defects which are found in psoriasiform dermatitis may be improved if TRPV1 is genetically ablated. We suggest that TRPV1 should be further

investigated as a candidate target for psoriatic skin disease in humans.

Conflict of interest

The authors state no conflict of interest.

Acknowledgements

References

[1] A. Chiricozzi, P. Romanelli, E. Volpe, G. Borsellino, M. Romanelli, Scanning the Immunopathogenesis of Psoriasis, Int J Mol Sci 19(1) (2018).

[2] A. Saggini, S. Chimenti, A. Chiricozzi, IL-6 as a druggable target in psoriasis: focus on pustular variants, J Immunol Res 2014 (2014) 964069.

[3] J. Giang, M.A.J. Seelen, M.B.A. van Doorn, R. Rissmann, E.P. Prens, J. Damman, Complement Activation in Inflammatory Skin Diseases, Front Immunol 9 (2018) 639.

[4] H.J. Hunter, C.E. Griffiths, C.E. Kleyn, Does psychosocial stress play a role in the exacerbation of psoriasis?, Br J Dermatol 169(5) (2013) 965-74.

[5] P.C. Arck, A. Slominski, T.C. Theoharides, E.M. Peters, R. Paus, Neuroimmunology of stress: skin takes center stage, J Invest Dermatol 126(8) (2006) 1697-704.

[6] J.E. Choi, A. Di Nardo, Skin neurogenic inflammation, Semin Immunopathol 40(3) (2018) 249-259.

[7] L. Riol-Blanco, J. Ordovas-Montanes, M. Perro, E. Naval, A. Thiriot, D. Alvarez, S. Paust, J.N. Wood, U.H. von Andrian, Nociceptive sensory neurons drive interleukin-23-mediated psoriasiform skin inflammation, Nature 510(7503) (2014) 157-61.

[8] F. Cevikbas, X. Wang, T. Akiyama, C. Kempkes, T. Savinko, A. Antal, G. Kukova, T. Buhl, A. Ikoma, J. Buddenkotte, V. Soumelis, M. Feld, H. Alenius, S.R. Dillon, E. Carstens, B. Homey, A. Basbaum, M. Steinhoff, A sensory neuron-expressed IL-31 receptor mediates T helper cell-dependent itch: Involvement of TRPV1 and TRPA1, J Allergy Clin Immunol 133(2) (2014) 448-60.

[9] J.G. Bonchak, R.A. Swerlick, Emerging therapies for atopic dermatitis: TRPV1 antagonists, J Am Acad Dermatol 78(3S1) (2018) S63-S66.

[10] W.X. Huang, F. Yu, R.M. Sanchez, Y.Q. Liu, J.W. Min, J.J. Hu, N.B. Bsoul, S. Han, J. Yin, W.H. Liu, X.H. He, B.W. Peng, TRPV1 promotes repetitive febrile seizures by pro-inflammatory cytokines in immature brain, Brain Behav Immun 48 (2015) 68-77.

[11] O. Gouin, K. L'Herondelle, N. Lebonvallet, C. Le Gall-Ianotto, M. Sakka, V. Buhe, E. Plee-Gautier, J.L. Carre, L. Lefeuvre, L. Misery, R. Le Garrec, TRPV1 and TRPA1 in cutaneous neurogenic and chronic inflammation: pro-inflammatory response induced by their activation and their sensitization, Protein Cell 8(9) (2017) 644-661.

[12] D. Roblin, G. Yosipovitch, B. Boyce, J. Robinson, J. Sandy, V. Mainero, R. Wickramasinghe, U. Anand, P. Anand, Topical TrkA Kinase Inhibitor CT327 is an Effective, Novel Therapy for the Treatment of Pruritus due to Psoriasis: Results from Experimental Studies, and Efficacy and Safety of CT327 in a Phase 2b Clinical Trial in Patients with Psoriasis, Acta Derm Venereol 95(5) (2015) 542-8.

[13] L.A. Nattkemper, H.L. Tey, R. Valdes-Rodriguez, H. Lee, N.K. Mollanazar, C. Albornoz, K.M. Sanders, G. Yosipovitch, The Genetics of Chronic Itch: Gene Expression in the Skin of Patients with Atopic Dermatitis and Psoriasis with Severe Itch, J Invest Dermatol 138(6) (2018) 1311-1317.

[14] L. Pecze, P. Pelsoczi, M. Kecskes, Z. Winter, A. Papp, K. Kaszas, T. Letoha, C. Vizler, Z. Olah, Resiniferatoxin mediated ablation of TRPV1+ neurons removes TRPA1 as well, Can J Neurol Sci 36(2) (2009) 234-41.

[15] D. Mohammed, P.J. Matts, J. Hadgraft, M.E. Lane, Depth profiling of stratum corneum biophysical and molecular properties, Br J Dermatol 164(5) (2011) 957-65.

[16] S.R. Feldman, D.M. Bushnell, P.A. Klekotka, M. Scanlon, M.L. Martin, S.W. Wade, W. Yang, L. Pinto, L. Kircik, H.N. Viswanathan, Differences in psoriasis signs and symptom severity between patients with clear and almost clear skin in clinical practice, J Dermatolog Treat 27(3) (2016) 224-7.

[17] Y. Lee, Y.J. Je, S.S. Lee, Z.J. Li, D.K. Choi, Y.B. Kwon, K.C. Sohn, M. Im, Y.J. Seo, J.H. Lee, Changes in transepidermal water loss and skin hydration according to expression of aquaporin-3 in psoriasis, Ann Dermatol

24(2) (2012) 168-74.

[18] A. Kemeny, X. Kodji, S. Horvath, R. Komlodi, E. Szoke, Z. Sandor, A. Perkecz, C. Gyomorei, G. Setalo, B. Kelemen, T. Biro, B.I. Toth, S.D. Brain, E. Pinter, R. Gyulai, TRPA1 Acts in a Protective Manner in Imiquimod-Induced Psoriasiform Dermatitis in Mice, J Invest Dermatol 138(8) (2018) 1774-1784.

[19] M. Liu, X. Li, X.Y. Chen, F. Xue, J. Zheng, Topical application of a linoleic acid-ceramide containing moisturizer exhibit therapeutic and preventive benefits for psoriasis vulgaris: a randomized controlled trial, Dermatol Ther 28(6) (2015) 373-82.

[20] R. Darlenski, E. Hristakieva, U. Aydin, D. Gancheva, T. Gancheva, A. Zheleva, V. Gadjeva, J.W. Fluhr, Epidermal barrier and oxidative stress parameters improve during in 311nm narrow band UVB phototherapy of plaque type psoriasis, J Dermatol Sci 91(1) (2018) 28-34.

[21] J.W. Yun, J.A. Seo, Y.S. Jeong, I.H. Bae, W.H. Jang, J. Lee, S.Y. Kim, S.S. Shin, B.Y. Woo, K.W. Lee, K.M. Lim, Y.H. Park, TRPV1 antagonist can suppress the atopic dermatitis-like symptoms by accelerating skin barrier recovery, J Dermatol Sci 62(1) (2011) 8-15.

[22] B.E. Kim, M.D. Howell, E. Guttman-Yassky, P.M. Gilleaudeau, I.R. Cardinale, M. Boguniewicz, J.G. Krueger, D.Y. Leung, TNF-alpha downregulates filaggrin and loricrin through c-Jun N-terminal kinase: role for TNF-alpha antagonists to improve skin barrier, J Invest Dermatol 131(6) (2011) 1272-9.

[23] S. Bertin, Y. Aoki-Nonaka, P.R. de Jong, L.L. Nohara, H. Xu, S.R. Stanwood, S. Srikanth, J. Lee, K. To, L. Abramson, T. Yu, T. Han, R. Touma, X. Li, J.M. Gonzalez-Navajas, S. Herdman, M. Corr, G. Fu, H. Dong, Y. Gwack, A. Franco, W.A. Jefferies, E. Raz, The ion channel TRPV1 regulates the activation and proinflammatory properties of CD4(+) T cells, Nat Immunol 15(11) (2014) 1055-1063.

[24] S. Mashiko, S. Bouguermouh, M. Rubio, N. Baba, R. Bissonnette, M. Sarfati, Human mast cells are major IL-22 producers in patients with psoriasis and atopic dermatitis, J Allergy Clin Immunol 136(2) (2015) 351-9 e1.

[25] S.P. Jin, S.J. Koh, D.A. Yu, M.W. Kim, H.T. Yun, D.H. Lee, H.S. Yoon, S. Cho, H.S. Park, Imiquimod-applied Interleukin-10 deficient mice better reflects severe and persistent psoriasis with systemic inflammatory state, Exp Dermatol 27(1) (2018) 43-49.

Figure legends

Fig. 1. PSI and TEWL scores of IMQ-induced PsD. (A) Representative images are shown of the back skin of vehicle- and IMQ-treated in WT mice vs. TRPV1 KO mice on Day 5. (B) The PSI scores, including scales (S), erythema (E), induration (I) are shown in mice treated with IMQ for 5 days in WT and TRPV1 KO group, respectively. (C) TEWL Scores in WT or TRPV1 KO mice with IMQ treatment are shown after 5 days (n=12 in each vehicle group, n=13 in each IMQ group). P-value scores for PSI scores were calculated by student T test; TEWL scores by one-way ANOVA followed by post-hoc comparison (Tukey's HSD) test. Mean ± S.D. values are indicated (*P<0.05, **P<0.01, ****P<0.0001).
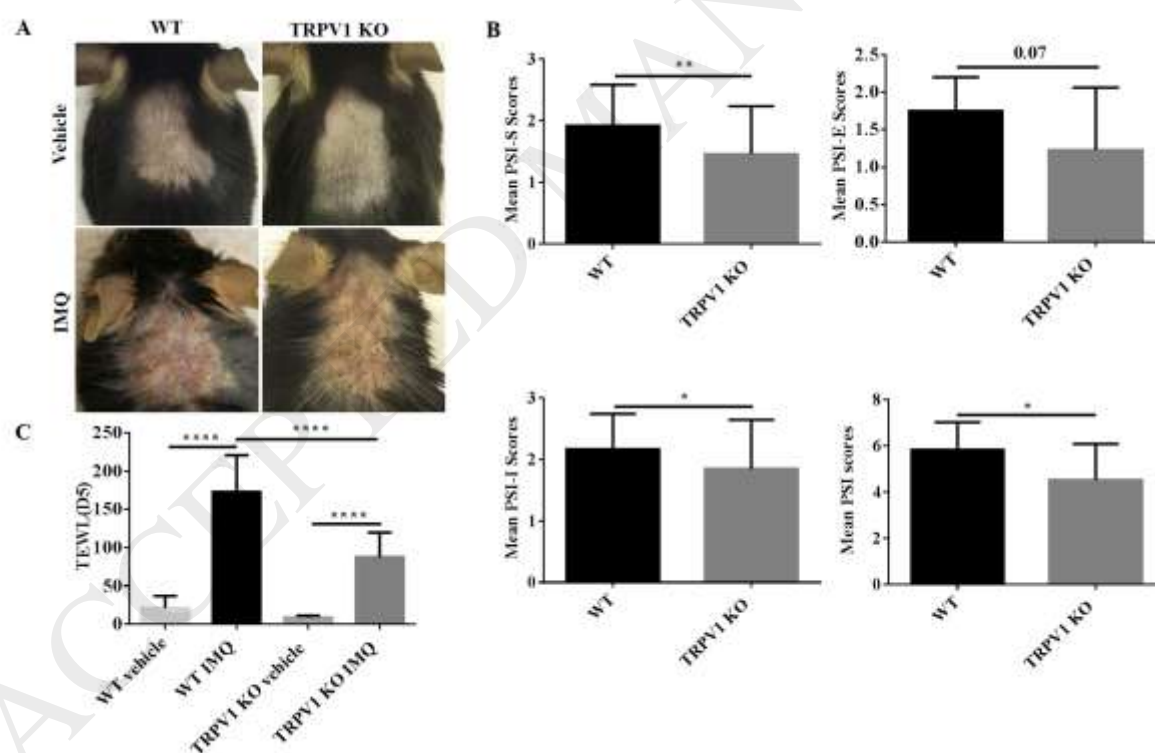
Fig. 2. Epidermal hyperplasia, pSTAT3, and Ki67 expression following IMQ treatment. (A) After IMQ treatment for 5 days, histopathology showed significant changes in hyperkeratosis and acanthosis in WT vs. TRPV1 KO mice. 200X magnification (scale bar=50μm). (B) Quantitative epidermal thickness changes are shown for TRPV1 KO mice and WT mice (n=12 in each vehicle group, n=13 in each IMQ group). (C) Ki67 staining and quantification per microscopic field (200X) are shown from treated skin of TRPV1 KO vs. WT mice treated with IMQ/vehicle (5 days of treatment), n=6 in each group. (D) pSTAT3 staining and quantification per microscopic field (200X) are shown from treated skin of TRPV1 KO vs. WT mice treated with IMQ/vehicle (5 days of treatment), n=6 in each group (scale bar=50μm). Statistical testing included student T test for epidermal thickness, one-way ANOVA followed by post-hoc comparison (Tukey's HSD) test for Ki67 and pSTAT3 analysis. Mean ± S.D. values are indicated (*P<0.05, **P<0.01, ****P<0.0001).
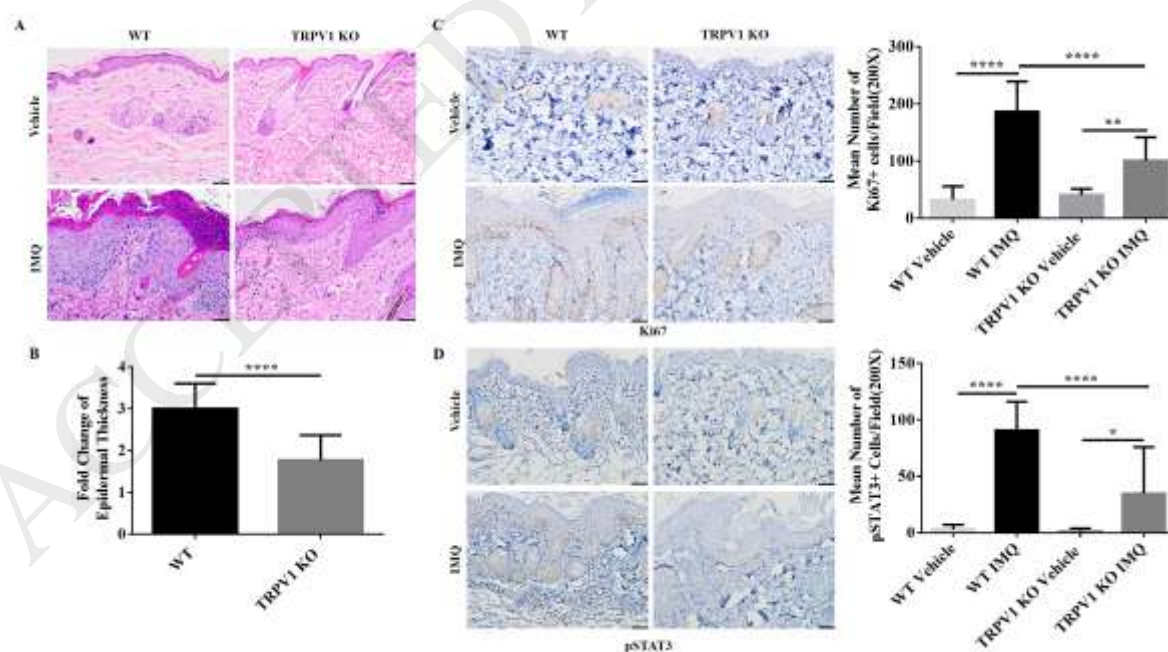
Fig. 3. Changes in angiogenesis and MM formation in WT and TRPV1 KO mice. (A) Skin

sections from indicated mice were stained with anti-CD31 antibodies as described in

Materials and Methods and (B) quantified with respect to mean vessel density (MVD) (n=6 in

each group). (C) MM in IMQ-treated skin from WT and TRPV1 KO mice were (D) quantified

in terms of relative MM area after 5 days of IMQ or vehicle treatment (n=12 in each vehicle

group, n=13 in each IMQ group) in TRPV1 KO mice/WT mice treated with IMQ/vehicle for

5 days. (200X magnification; scale bar=50μm). Statistical analysis included a student T test

for MM and one-way ANOVA followed by post-hoc comparison (Tukey's HSD) test for

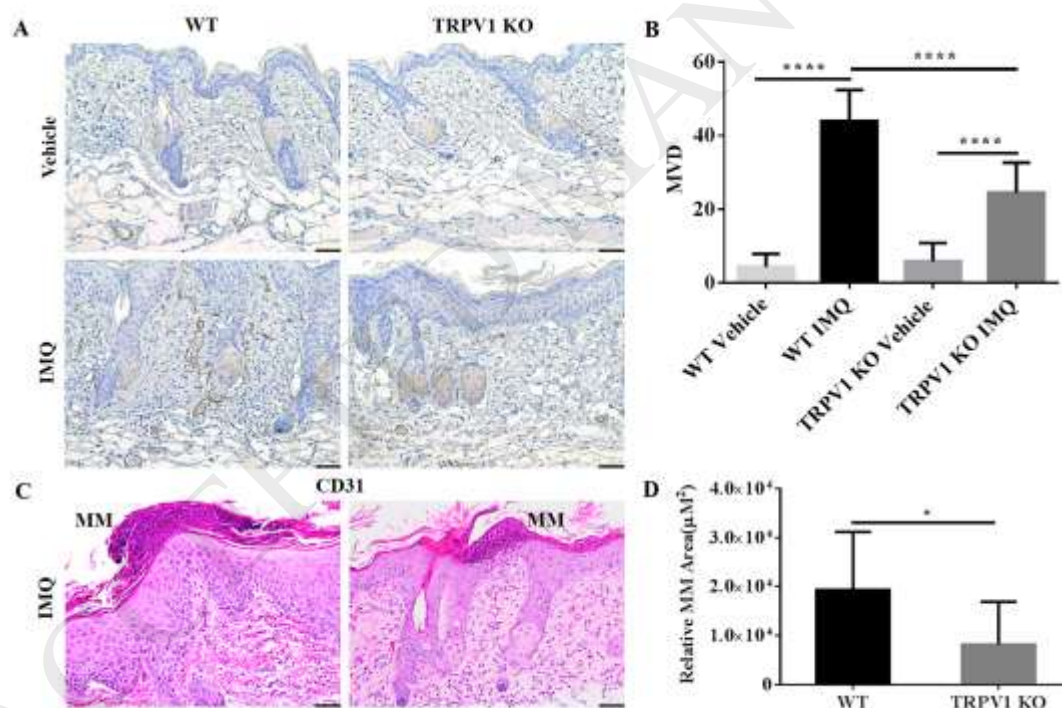MVD. Mean ± S.D. values are indicated (*P<0.05, ****P<0.0001).

Fig. 4. Immunohistochemical and Giemsa staining of IMQ- and vehicle-treated skin in WT and TRPV1 KO mice. (A) CD45 staining and quantification per HPF (400X). (B) CD3+ T cell staining and quantification and (C) mast cell staining with Giemsa and quantification after 5 days of treatment. Representative photos are shown with 200X magnification (scale bar=50μm). Statistical analysis was performed using one-way ANOVA followed by post-hoc comparison (Tukey's HSD) test. N=6 per group. Mean ± S.D. values are indicated (*P<0.05, ****P<0.0001).
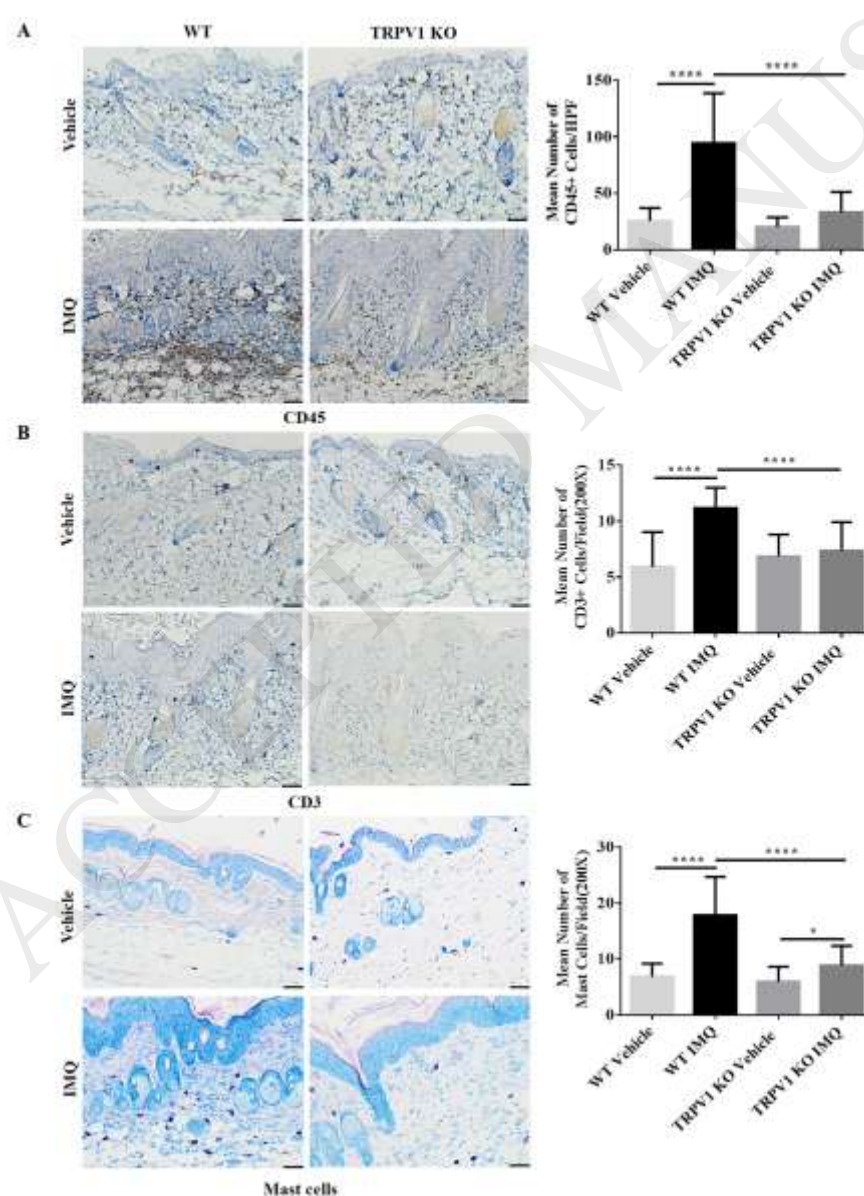
Fig. 5. Transcriptional mRNA expression of psoriasis-related genes in lesions. Relative skin

mRNA expression of IL-1β, IL-6, IL-23, S100A8, CXCL1, IL-10, IL-17A/F, IL-22 and

DEFB4 in TRPV1 KO mice/WT mice treated with IMQ/vehicle were analyzed and quantified

as indicated in Material and Methods. Results are normalized to GAPDH expression. N=12 in

each vehicle group, n=13 in each IMQ group. Statistical analysis was performed using one-

way ANOVA followed by post-hoc comparison (Tukey's HSD) test. Mean ± S.D. values are

indicated (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001).