AUTHOR CONTRIBUTIONS

Conceptualization: LLC, ES; Data Curation: ES; Formal Analysis: SA, LLC, ES; Methodology: SA, LLC, ES; Project Administration: LLC, ES; Resources: LLC, ES; Software: SA, LLC, ES; Supervision: LLC, ES; Validation: LLC, ES; Writing - Original Draft Preparation: SA, LLC, ES; Writing - Review and Editing: SA, LLC, ES

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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.2022.10.024.

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TRPA1 Mediates Contact Hypersensitivity Induced by 2,4-Dinitrochlorobenzene

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JID Open

TO THE EDITOR

Allergic contact dermatitis (ACD) is an increasing health problem with limited treatment options. In this study, we describe our recent findings showing that TRPA1 mediates DNCB-induced contact dermatitis in experimental models. The results support TRPA1 as a drug target to prevent/treat ACD.

The compound 2,4-dinitrocholorobenzene (DNCB) is a known contact sensitizer and a potent cause of ACD. It is a precursor molecule in industrial synthesis and a widely used tool to study contact hypersensitivity. Almost every subject with an intact immune system is sensitized to DNCB after sufficient exposure (Friedmann et al., 1983). When applied to the skin, DNCB acts as an irritant and as a hapten. The recognition of DNCB–skin protein complexes by Langerhans cells and secretion of cytokines by injured keratinocytes induce immunization (Ho et al., 2015). Memory T cells are thus formed, and re-exposure to DNCB elicits an immune response. Both type 1 and type 2 T helper (Th) lymphocyte (Th1 and Th2)-mediated responses are involved in DNCB-induced hypersensitivity (Gittler et al., 2013; Martin, 2015).

TRPA1 is a nonselective cation channel expressed most famously in sensory nerves and also in immune cells and some other non-neuronal cells (Atoyan et al., 2009; Naert et al., 2021; Szabó et al., 2022). It is opened by irritating exogenous compounds such as allyl isothiocyanate from mustard oil or by endogenous inflammatory factors such as reactive oxygen and nitrogen species (Mahajan et al., 2021). In addition to causing itch and pain, TRPA1 activation is known to cause neurogenic inflammation by

Abbreviations: ACD, allergic contact dermatitis; DNCB, 2,4-dinitrocholorobenzene; Th, T helper; TRPA1, transient receptor potential ankyrin 1

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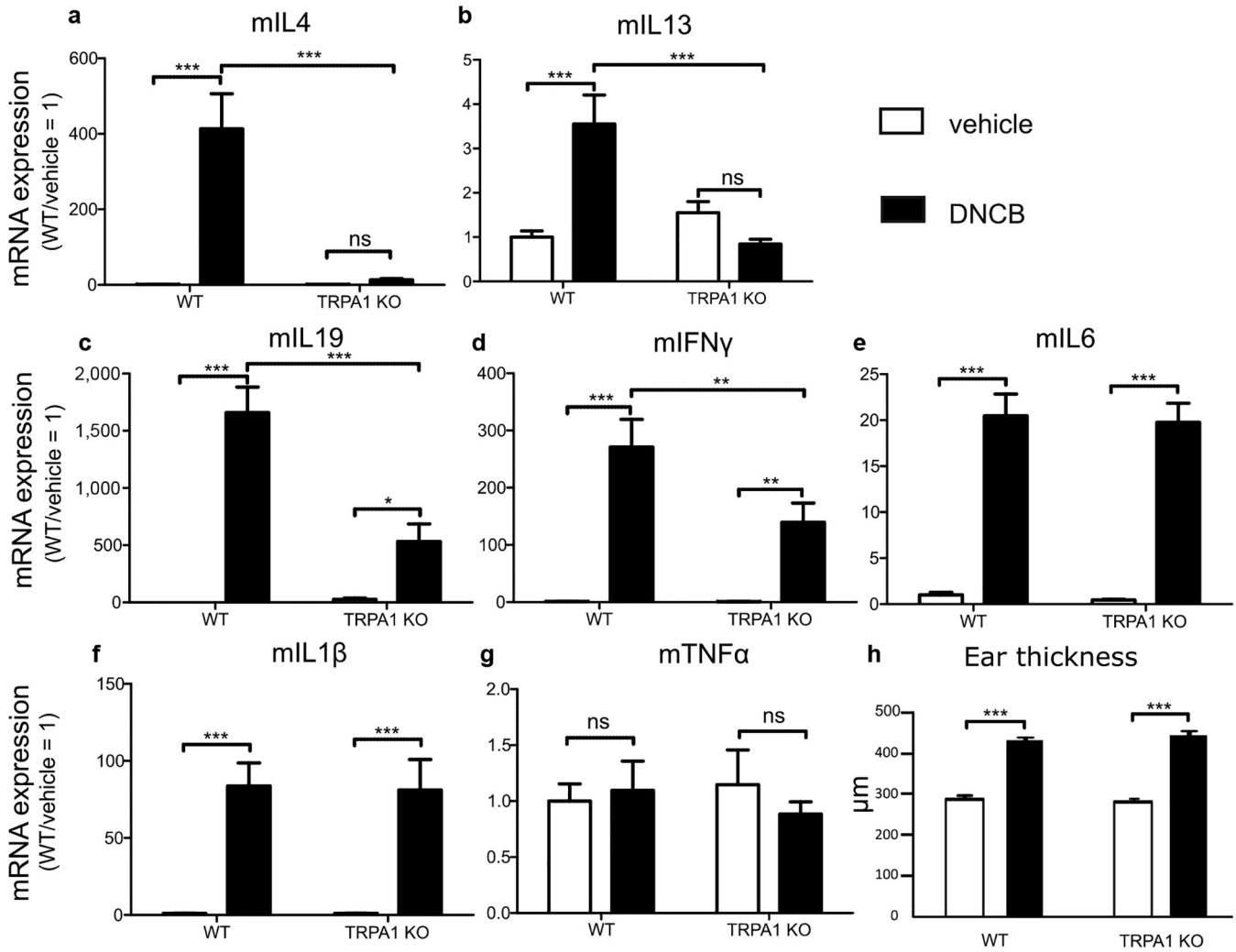

Figure 1. The expression of Th1 and particularly Th2-related cytokines was lower in DNCB-challenged ear skin in TRPA1-deficient mice than in wild-type mice in the experimental model of DNCB-induced contact hypersensitivity. Twenty-five μl of 3% DNCB in 4:1 acetone:olive oil was applied to the shaved abdomen of TRPA1-deficient (KO) and wild-type mice on days 1 and 3 of the experiment to sensitize the mice to DNCB. On day 6, mice were challenged with DNCB applied to their right ears. A vehicle solution of acetone:olive oil was applied to the left ear. After 24 hours, the ear samples were collected. RT-qPCR was used to measure the expression of the genes (**a**) *IL4*, (**b**) *IL13*, (**c**) *IL19*, (**d**) *IFNγ*, (**e**) *IL6*, (**f**) *IL1β*, and (**g**) *TNFα*. Ear thickness is portrayed in **h**. The results are presented as the mean + SEM (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. DNCB, 2,4-dinitrocholorobenzene; KO, knockout; mIFNγ, mouse IFNγ; mIL, mouse IL; ns, not significant; Th, T helper; WT, wild-type.

inducing the release of substance P and calcitonin gene-related peptide (Koivisto et al., 2018; Zygmunt and Högestätt, 2014).

DNCB has been reported to activate TRPA1 (Saarnilehto et al., 2014; Wu et al., 2022); therefore, we hypothesized that TRPA1 might have a role in DNCB-induced contact hypersensitivity. It is of interest that other contact sensitizers such as oxazolone, p-benzoquinone, and isoeugenol also activate TRPA1 (Anand et al., 2008; Ibarra and Blair, 2013; Liu et al., 2013).

In this study, we aimed to assess the role of TRPA1 in DNCB-induced inflammatory skin reactions in experimental in vivo models of acute inflammatory edema and ACD.

Using Fluo 3-AM intracellular calcium measurements, we showed in vitro that DNCB is a potent TRPA1 agonist (Supplementary Figure S1a and b). Accordingly, DNCB-induced inflammatory paw edema in mice was blocked by genetic and pharmacologic inhibition of TRPA1 (Supplementary Figure S1c).

In the experimental model of contact hypersensitivity, DNCB-sensitized TRPA1-deficient mice were found to have decreased local expression of cytokines IL-4, IL-13, IL-19, and IFN-γ after DNCB challenge compared with that of wild-type mice (Figure 1). Cytokines associated with both Th1 and Th2-type responses were downregulated, but the effect was more obvious in Th2 cytokine expression. TRPA1-deficient mice had virtually no DNCB-induced upregulation of IL-4 or IL-13. Accordingly, a recent study showed that TRPA1-deficient mice had lower Th2 cytokine expression in DNCB-induced atopic dermatitis (Zeng et al., 2021).

In the mechanistic studies, the flow cytometry results showed that similar to the glucocorticoid dexamethasone (used as a control treatment), the TRPA1 antagonist HC-030031 decreased the expression of CD54 and CD86 on THP-1 cells after exposure to

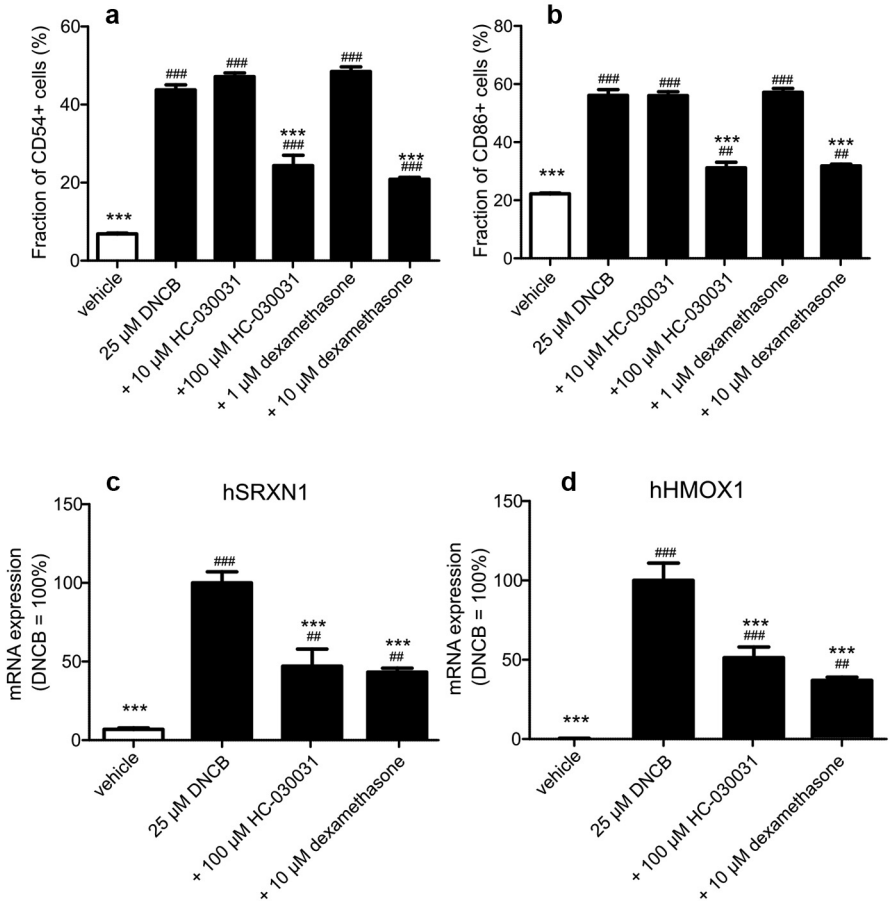

Figure 2. TRPA1 antagonist treatment inhibits hypersensitivity-related phenomena in monocytes and keratinocytes in vitro. (**a, b**) The TRPA1 antagonist HC-030031 attenuated the activation of DNCB-challenged THP-1 monocytes into antigen-presenting cells as measured by flow cytometry. (**c, d**) Expression of the sensitizer marker genes *SRXN1* and *HMOX1* was downregulated by treatment with the TRPA1 antagonist HC-030031 in HaCaT keratinocytes. The results are presented as the mean + SEM (n = 6). $^{***}P < 0.001$ compared with the 25 μM DNCB-treated column. $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared with the vehicle-treated column. DNCB, 2,4-dinitrochlorobenzene; hHMOX1, human HMOX1; hSRXN1, human SRXN1.

DNCB (Figure 2a and b). This result indicates that treatment with HC-030031 reduces the transformation of THP-1 monocytes to antigen-presenting cells. This suggests that TRPA1 might have a role in sensitization to the contact antigen. In this setting, only THP-1 cells were present. Therefore, TRPA1 should be expressed by THP-1 cells themselves. In support, it has been shown that TRPA1 is expressed in primary human monocytes and in the macrophages of the gut of patients with Crohn's Disease (Billeter et al., 2015; Kun et al., 2014), and on the basis of our preliminary data, TRPA1 is also expressed in THP-1 cells.

We also investigated how HC-030031 treatment alters the expression of sensitizer marker genes in HaCaT keratinocytes (van der Veen et al., 2015, 2013), which have been shown to express TRPA1 under inflammatory conditions (Luostarinen et al., 2021). The expression of two genes, namely, *SRXN1* and *HMOX1*, both related to oxidative stress, was downregulated by HC-030031 (Figure 2c and d).

We hypothesize that TRPA1 activation by hapten molecules such as DNCB might provoke the immune system to recognize hapten–protein complexes as antigens. This is supported by a previous study showing that TRPA1-activating phthalate esters and other non-electrophilic TRPA1 agonists enhanced dendritic cell trafficking and skin sensitization to FITC in a TRPA1-mediated manner in mice (Shiba et al., 2012). The cinnamon-derived TRPA1 agonist cinnamaldehyde (Zygmunt and Högestätt, 2014) has also been reported to cause ACD (Attasuriyanan et al., 2019; Guarneri, 2010; Isaac-Renton et al., 2015; Lauriola et al., 2010). Cinnamaldehyde also promotes sensitization to FITC when given simultaneously (Shiba et al., 2012). Therefore, we propose that TRPA1 antagonists could prevent/treat sensitization to common contact sensitizers.

Further studies are needed to understand the mechanisms of how TRPA1 promotes hypersensitivity/ACD. Candidate mediators include neuroinflammatory transmitters such as calcitonin gene-related peptide and substance P. Calcitonin gene-related peptide seems to have an interesting two-way role in contact hypersensitivity because it has been shown to inhibit Th1-type contact hypersensitivity but to promote Th2-type disease (Kerage et al., 2019; Mikami et al., 2011). It is also of interest that neuronal TRPV1 activation skews dendritic cells toward Th2-mediated allergic immune response through the release of substance P (Perner et al., 2020). TRPA1 and TRPV1 are largely coexpressed in neurons, and their functions are widely interlinked (Fernandes et al., 2012).

In conclusion, we showed that DNCB is a potent TRPA1 agonist. TRPA1 deficiency attenuated the expression of Th1 and more so Th2 inflammatory cytokines in DNCB-induced contact hypersensitivity in mice. DNCB-induced monocyte activation to antigen-presenting cells was attenuated by TRPA1 antagonist treatment, as was the expression of the DNCB-induced oxidative stress–related genes *SRXN1* and *HMOX1* in keratinocytes. We hypothesize that TRPA1 activation is a contributing factor in the allergic contact sensitization process and that TRPA1 activation makes haptens, such as DNCB, more potent sensitizers. The results support TRPA1 antagonists as drugs with the potential to prevent and/or treat ACD.

Ethics statement

Animal experiments were approved by the Animal Experimental Board in Finland and carried out in accordance with the legislation for the protection of animals used for scientific purposes (grant numbers ESAVI/1258/2018 and ESAVI/24887/2020, directive 2010/63/EU).

Data availability statement

The datasets supporting the conclusions of this article are included within the article and supplementary material.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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Conceptualization: IMO, MH, LJM, ISJ, LL, EM; Funding Acquisition: EM; Investigation: IMO, MH, HS, TL, LK, EM; Formal Analysis: IMO, MH, HS; Methodology: IMO, MH, LJM, ISJ, LL, EM; Project Administration: EM; Supervision: EM; Writing - Original Draft Preparation: IMO; Writing - Review and Editing: MH, LJM, HS, TL, LK, ISJ, LL, EM

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SUPPLEMENTARY MATERIAL

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Spatial Randomness in the Distribution of Acquired Melanocytic Nevi of the Back in a Population-Based Sample

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TO THE EDITOR

It is assumed that body sites may differ in their susceptibility to melanocytic proliferation and malignant transformation (Green, 1992). Identifying areas where there is a high density (or cluster) of nevi may aid in the early detection of melanoma. Recently, in a high-risk melanoma cohort (n = 313; age range = 30–49 years), Chousakos et al. (2022) assessed whether the distribution of melanocytic neoplasms (melanocytic nevi and melanomas) on the back followed the paradigm of complete spatial randomness. They first calculated a patient-level Clark–Evans score (Z_k or standardized Clark–Evans aggregation index [Clark and Evans, 1954]) to assess the complete spatial randomness of the distribution of melanocytic neoplasms >5 mm in diameter within each participant, where $Z_k = 0$ indicates complete spatial randomness, $Z_k < 0$ indicates clustering, and $Z_k > 0$ indicates dispersion (a visual example is provided in Supplementary Figure S1). They then conducted a lesion-level analysis to assess the spatial distribution of each lesion compared with that of other lesions over the area of interest. The nearest neighbor distance (denoted as D_r) for each lesion was calculated and compared with the average nearest neighbor values (denoted as D_C and D_D, respectively) that would have resulted in a Clark–Evans score of either $Z_k = -1.96$ (clustering) and $Z_k = 1.96$ (dispersion) to classify each lesion as proximal ($D_r < D_C$), distant ($D_r > D_D$), or neither ($D_c < D_r < D_D$) (see Supplementary Materials and Methods). Overall, on the basis of the mean Z_k of −0.55, they observed that the spatial distribution of melanocytic neoplasms deviated toward clustering, as opposed to complete spatial randomness or dispersion ($P < 0.001$). However, in the patient-level analysis, the distribution of only a few patients (11%) was classified as significantly clustered. In the lesion-level analysis, 79% of melanomas, compared with 45% of nevi, appeared more frequently in proximity to neighboring neoplasms.

In the study described in this paper, we used data from 152 participants (aged 27–73 years) from a longitudinal general population–based study (Jayasinghe et al., 2023) (protocol is described previously [Koh et al., 2018]) of three-dimensional total body imaging to replicate methods described in the study by Chousakos et al. (2022) in a population-based sample. This study was reviewed and approved by the Human Research Ethics Committees of Metro South Health (Brisbane, Australia) (HREC/16/QPAH/125), The University of Queensland (Brisbane, Australia) (2016000554), Queensland University of Technology (Brisbane, Australia) (1600000515), and QIMR Berghofer Medical Research Institute (Herston, Australia) (P2271). Written, informed consent was taken from all participants.

Nevi >5 mm diameter were identified using a combination of a validated automated algorithm (Betz-Stablein et al., 2022) embedded in the Vectra WB 360 software (Canfield Scientific, Parsippany, NJ) and dermatologist's assessment (explained in detail in the Supplementary Materials and Methods). The surface area of the back was obtained by the Mirror DermaGraphix body mapping software (Canfield Scientific) according to Chousakos et al. (2022)'s anatomical boundaries.

Because our study cohort was ascertained from the general population as opposed to ascertaining from a high-risk population, of the 152 participants included in our study, only 44 were identified as having three or more nevi ≥5 mm in diameter on the back. Thirty-two of the 44 participants (73%) were male, with a median age of 60 years (interquartile range = 50–65), and four had a history of melanoma. Overall, we had 288 melanocytic nevi (median = 5 per participant; interquartile range = 3–8) available for analysis, contrasting to the dataset in the study by Chousakos et al. (2022), consisting of nevi and melanoma.

In our overall participant-level analysis, the mean Clark–Evans score of $Z_k = -0.28$ (95% confidence interval = −0.69, 0.22) was higher than the mean $Z_k = -0.55$ (95% confidence interval = −0.69, −0.42) in Chousakos et al. (2022)'s study. Moreover, our participants' scores were positively skewed (Supplementary Figure S2) compared with the Gaussian distribution observed

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SUPPLEMENTARY MATERIALS AND METHODS

Reagents

Reagents were provided by Sigma-Adrich (St. Louis, MO), if not indicated otherwise.

Cell culture

Human embryonic kidney 293 cells (ATCC, Manassas, VA) were cultured in EMEM (Lonza, Verviers, Belgium) at 37 °C in 5% carbon dioxide. EMEM was supplemented with fetal bovine serum (10%), sodium pyruvate (1 mM), sodium bicarbonate (1.5%), nonessential amino acids (0.1 mM each, all from Lonza), streptomycin (100 μg/ml), penicillin (100 IU/ml), and amphotericin B (250 ng/ml, all from Gibco, Thermo Fisher Scientific, Waltham, MA). Cells were cultured on 96-well plates (0.03×10^6/well) and transiently transfected with human *TRPA1* (0.2 μg/well, Origene, Rockville, MD) or mouse *Trpa1* (0.2 μg/well, GeneCopoeia, Rockville, MD) plasmid using Lipofectamine 2000 (0.5 μl/well, Invitrogen, Paisley, UK) 20 hours before the experiments were started.

HaCaT keratinocytes (Boukamp et al., 1988) from Cell Lines Service (Eppelheim, Germany) were cultured at 37 °C in 5% carbon dioxide in DMEM supplemented with 0.1 mM nonessential amino acids, fetal bovine serum (10%) (all from Lonza), streptomycin (100 μg/ml), penicillin (100 IU/ml), and amphotericin B (250 ng/ml, all from Gibco). Cells were cultured on 24-well plates (0.2×10^6/well) and grown for 72 hours before the experiments.

THP-1 monocytes (ATCC) were cultured at 37 °C in 5% carbon dioxide in RPMI 1640 medium supplemented with fetal bovine serum (10%), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (10 mM), and sodium pyruvate (1 mM; all from Lonza); sodium bicarbonate (1.5 mg/ml; Gibco), streptomycin (100 μg/ml), penicillin (100 IU/ml), and amphotericin B (250 ng/ml, all from Gibco); and glucose (2.5 mg/ml) and 2-mercaptoethanol (0.05 mM). Cells were seeded on 24-well plates (1×10^6/well) and cultured with the compounds of interest.

Intracellular calcium measurements

Fluo 3-AM protocol was used to measure 2,4-dinitrochlorobenzene (DNCB)-induced calcium-influx in TRPA1-transfected human embryonic kidney 293 cells. Transiently transfected human embryonic kidney 293 cells were loaded with Fluo 3-AM ester (2.5 μM Fluo 3-AM in Hanks' Basic Salt Solution [Lonza], pH 7.45, with 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 1 mg/ml BSA, 2.5 mM probenecid, and 0.08% Pluronic F-127) for 40 minutes at room temperature. Subsequently, the cells were washed, buffer solution containing the TRPA1 antagonist HC-030031 or vehicle was added to the wells, and the cells were incubated at 37 °C for 30 minutes. Victor^3 1420 multi-label counter (PerkinElmer, Waltham, MA) at wavelength emission/excitation of 485/535 nm was used to measure free intracellular calcium concentrations. First, the background fluorescence was measured for 30 seconds before adding the TRPA1 agonist allyl isothiocyanate (50 μM) or DNCB (1, 3, 10, or 50 μM), and the measurements were continued for 30 seconds. The results were normalized to background fluorescence, and the area under the curve during a response to DNCB or allyl isothiocyanate was calculated.

Flow cytometry

To examine the influence of TRPA1 on cellular mechanisms of immunological sensitization, flow cytometry was used to measure THP-1 cell activation by exposure to DNCB (25 μM). The expression of cell surface proteins CD54 and CD86 was quantified to evaluate the transformation of THP-1 monocytes into antigen-presenting cells. Briefly, after 24 hours of incubation with the compounds of interest, the cell suspension was collected, centrifuged (400*g*, 5 minutes, 4 °C), washed twice with BSA/PBS (1%), and blocked for 20 minutes on ice with human Fc receptor binding inhibitor (eBioscience, San Diego, CA). Thereafter, the cells were treated with FITC-labeled anti-CD54 (ICAM-I) and PerCP-eFluor 710–labeled anti-CD86 (B7-2) antibodies or with appropriate isotype controls (all from eBioscience) for 30 minutes on ice, washed twice, and resuspended to BSA/PBS (0.1%) and stored on ice in the dark until analyzed with flow cytometry using FACSCanto II (BD, Franklin Lakes, NJ). Data were analyzed with FlowJo software (Tree Star, Ashland, OR).

DNCB-challenged HaCaT cells and RT-qPCR analysis

We inventively used a protocol created to identify potential contact sensitizers (van der Veen et al., 2015, 2013) using human HaCaT keratinocytes. We investigated whether the expression of the genes, known to be upregulated by contact sensitizers, would be attenuated by a TRPA1 antagonist in HaCaT cells challenged with DNCB (25 μM). Cells were treated with the compounds of interest for 4 hours, and RNA was isolated from the cells with RNA GenElute Mammalian Total RNA Miniprep kit and transcribed to cDNA using Maxima First Strand cDNA synthesis kit (Thermo Fisher Scientific). qPCR was performed using TaqMan Universal Master Mix and ABI Prism 7500 sequence detection system (Applied Biosystems, Foster City, CA). The qPCR cycling parameters were incubation at 50 °C for 2 minutes, incubation at 95 °C for 10 minutes, and thereafter at 40 cycles of denaturation at 95 °C for 15 seconds and annealing and extension at 60 °C for 1 minute. Primer and probe sequences are shown in Supplementary Table S1. The $2^{-\Delta\Delta CT}$ method was used to calculate the expression levels of genes of interest, which were first normalized against *GAPDH* mRNA.

Animals

Wild-type and TRPA1-deficient male B6;129P-Trpa1$^{tm1\text{-}Kykw}$/J mice were provided by Charles River Laboratories (Sulzfield, Germany). They were used to investigate the effects of genetic ablation of TRPA1 in experimental models of DNCB-induced acute inflammatory edema and contact hypersensitivity. Mice were housed under standard conditions (12:12 hour day:night cycle, 22 ± 1 °C). Nutrition and water were provided freely. Animal experiments were approved by the Animal Experimental Board in Finland and carried out in accordance with the legislation for the protection of animals used for scientific purposes (grant numbers ESAVI/1258/2018 and

ESAVI/24887/2020, directive 2010/63/EU).

DNCB-induced acute inflammatory paw edema

We used classical mouse paw edema model to measure the acute inflammatory effect of DNCB. There were three groups of mice: vehicle-treated wild-type mice, wild-type mice treated with the TRPA1 antagonist HC-030031 (300 mg/kg given intragastrically 2 hours before DNCB injection), and the vehicle-treated group of TRPA1-deficient mice. The affected paw was injected with 50 μl of 15 mM DNCB, whereas the contralateral side was injected with vehicle only. The paw volume was measured right before and 3 and 6 hours after the DNCB injection using plethysmometer (Ugo Basile, Comerio, Italy). The results in each time point were calculated as the volume increase from baseline in the DNCB-affected paw subtracted by the respective increase in the contralateral vehicle-injected paw.

DNCB-induced contact hypersensitivity

To study the role of TRPA1 in contact hypersensitivity in vivo, wild-type and TRPA1-deficient mice were used in an experimental model of contact hypersensitivity induced by DNCB. Mice were sensitized to DNCB (25 μl of 3% DNCB in 4:1 acetone:olive oil) on the first and the third day of the experiment by applying DNCB topically on shaved abdomen. Three days later on day 6 of the experiment, mice were challenged by applying DNCB topically to their right ear and vehicle to the left. Ear skin samples were collected 24 hours later on day 7, and RNA was isolated using GenElute Mammalian Total RNA Miniprep kit with proteinase K and transcribed to cDNA using Maxima First Strand cDNA synthesis kit (Thermo Fisher Scientific). RT-qPCR was performed as described earlier. Primer and probe sequences are shown in Supplementary Table S1.

Statistical analysis

The results were analyzed with GraphPad Prism 5.02 for Windows (GraphPad Software, San Diego, CA) using one- and two-way ANOVA with Dunnett's or Bonferroni's multiple-comparison post-hoc test. The results are expressed as the mean + SEM with $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

SUPPLEMENTARY REFERENCES

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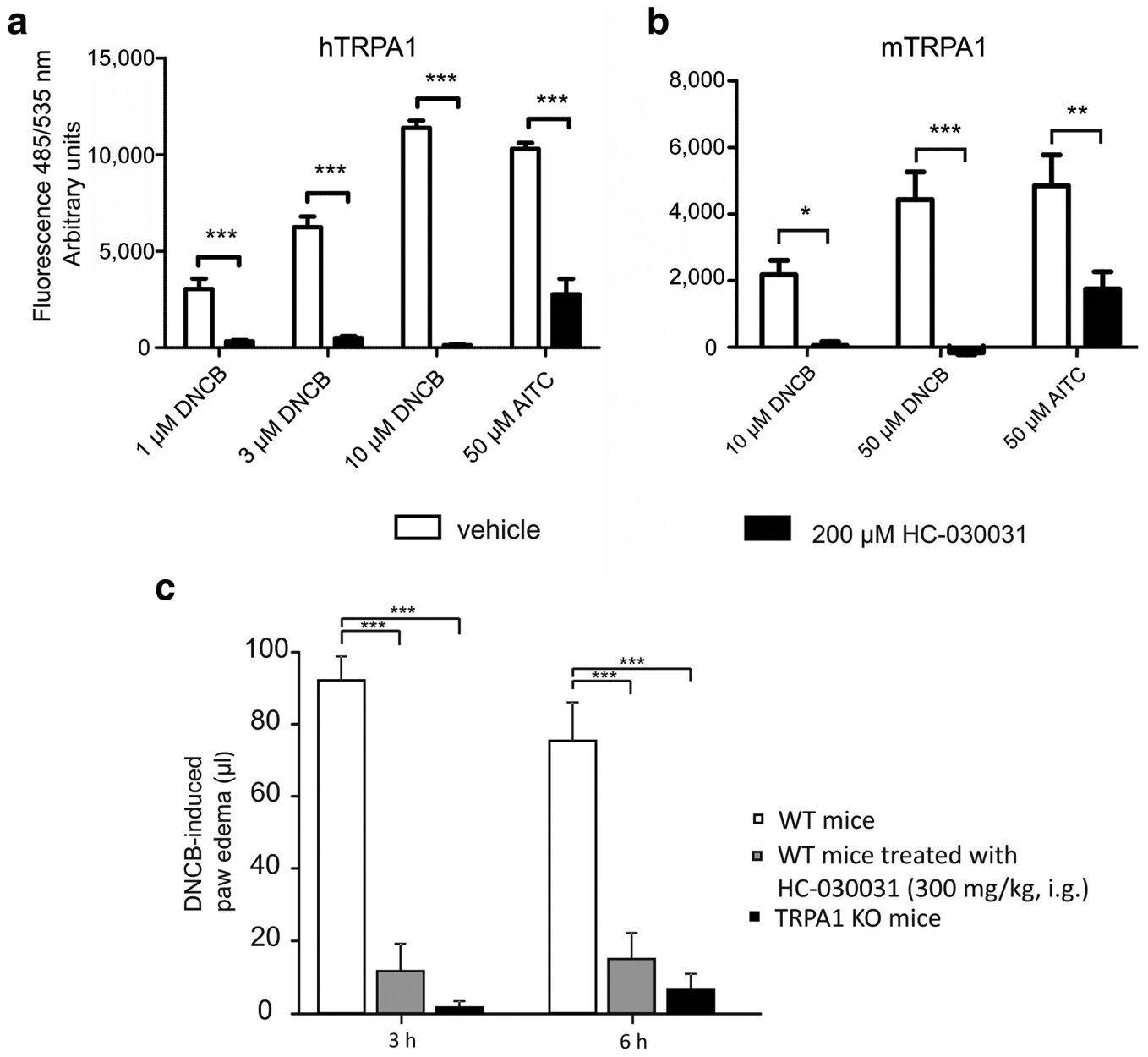

Supplementary Figure S1. TRPA1-agonistic features of DNCB in vitro and DNCB-induced inflammatory paw edema. (**a, b**) In Fluo 3-AM measurements, DNCB induced a TRPA1-mediated calcium influx in human embryonic kidney 293 cells transfected with a plasmid encoding (**a**) human and (**b**) mouse TRPA1. The Fluo 3-AM–loaded transfected human embryonic kidney 293 cells were preincubated for 30 minutes with the TRPA1 antagonist HC-030031 (200 μM) or vehicle solution before intracellular calcium measurement was started after dispensing of DNCB or allyl isothiocyanate to the cells. The fluorescence normalized to baseline was measured for 30 seconds, and the area under the curve during this time was calculated to represent a dynamic intracellular calcium concentration increase. Allyl isothiocyanate (50 μM) was used as a positive control. The results are presented as the mean + SEM of the area under the curve (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. **(c)** DNCB-induced paw edema was effectively attenuated by treatment with the TRPA1 antagonist HC-030031 and by genetic ablation of TRPA1. Mice were treated with the TRPA1 antagonist HC-030031 (300 mg/kg, i.g.) or its vehicle 2 hours before DNCB (15 mM) was injected into the right hind paw of each mouse and vehicle solution to the left. The volume of both hind paws was measured before and 3 and 6 hours after the DNCB application. The results in each time point were calculated as the volume change from baseline in the DNCB-injected paw subtracted by the respective change in the contralateral vehicle-injected paw. The results are presented as the mean + SEM (n = 6). $^{***}P < 0.001$. DNCB, 2,4-dinitrochlorobenzene; h, hour; hTRPA1, human TRPA1; i.g., intragastric; KO, knockout; mTRPA1, mouse TRPA1; WT, wild-type.

Supplementary Table S1. Primer and Probe Sequences and TaqMan Gene Expression Assays Used in this Study

Gene	Primer/Probe	Sequence/Assay ID
human *GAPDH*	Forward	AAGGTCGGAGTCAACGGATTT
	Reverse	GCAACAATATCCACTTTACCAGAGTTAA
	Probe	CGCCTGGTCACCAGGGCTGC
mouse *Gapdh*	Forward	GCATGGCCTTCCGTGTTC
	Reverse	GATGTCATCATACTTGGCAGGTTT
	Probe	TCGTGGATCTGACGTGCCGCC
mouse *Il6*	Forward	TCGGAGGCTTAATTACACATGTTC
	Reverse	CAAGTGCATCATCGTTGTTCATAC
	Probe	CAGAATTGCCATTGCACAACTCTTTTCTCA
mouse *Tnf*	Forward	GACCCTCACACTCAGATCATCTTCT
	Reverse	CCTCCACTTGGTGGTTTGCT
	Probe	AAAATTCGAGTGACAAGCCTGTAGCCCA
human *SRXN1*		Hs00607800_m1
human *HMOX1*		Hs01110250_m1
mouse *Il4*		Mm00445259_m1
mouse *Il13*		Mm00434204_m1
mouse *Il19*		Mm01288324_m1
mouse *Ifng*		Mm01168134_m1
mouse *Il1b*		Mm00434228_m1

Abbreviation: ID, identification.

Primer and probe sequences were obtained from Metabion GmbH (Planegg, Germany), and TaqMan gene expression assays from Thermo Fisher Scientific (Waltham, MA).