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Transient Receptor Potential Vanilloid 1 — a Polymodal Nociceptive Receptor — Plays a Crucial Role in Formaldehyde-Induced Skin Inflammation in Mice

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Abstract. Formaldehyde (FA) is irritating to the skin and is the main cause of sick building syndrome. However, the cutaneous reaction induced by long-term FA exposure has not been fully investigated. In our previous study, we demonstrated that repeated painting of 2% – 10% FA on mouse ears caused marked ear swelling and increased mRNA expression of transient receptor potential vanilloid 1 (TRPV1) and neurotrophins in the ear. TRPV1 is reported to be involved in neurogenic inflammation; therefore, in the present study, we investigated the role of TRPV1 in FA-induced skin inflammation using TRPV1 gene–knockout mice. Mice were painted with 5% FA once a week for 5 weeks, and ear swelling and mRNA expression were investigated. Ear swelling and increased expression of neurotrophins mRNA by FA provocation in wild-type mice were attenuated by disruption of the TRPV1 gene. Furthermore, painting with a threshold dose of capsaicin, which does not induce ear swelling in intact mice, caused marked ear swelling after painting the ear 5 times with FA, indicating that inflamed tissues after FA application are hypersensitive to various ligands of TRPV1 in mice. These results demonstrated that neurogenic inflammation via TRPV1 and neurotrophins could be involved in FA-induced dermatitis.

Keywords: ear swelling, formaldehyde, neurotrophin, transient receptor potential vanilloid 1

Introduction

During the 1980s, predominately in Europe and the United States, office works began feeling nauseous, showed signs of mucosal or skin irritation, and an increasing frequency of headaches (1). The World Health Organization (WHO) named these symptoms sick building syndrome (SBS) (2) and the name has since been used to describe symptoms that have no clear etiology and are attributed to a particular building environment. SBS shows a broad range of symptoms and many factors are responsible for its development or worsening conditions. The representative causative factors are a) physical environmental factors such as heat and humidity (3), b) biological factors such as fungus (4), and c) chemical factors including formaldehyde (FA), xylene, and toluene (5).

FA is a representative chemical factor of SBS. FA irritates the skin, eyes, and respiratory system (6). Furthermore, exposure to liquid FA likely leads to sensitization in humans (7). In addition, it is suggested that exposure to chemical substances including FA can induce allergic and neurogenic inflammation of the airway and skin (8 – 10); however, the effects of long-term FA exposure to the airway and skin have not been investigated in detail.

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Our previous study demonstrated that repeated painting of 2% – 10% FA on mouse ears induced marked ear swelling with significant infiltration of inflammatory leukocytes into the dermis and increased mRNA expression of interleukin-4 (IL-4), neurotrophins, and transient receptor potential vanilloid 1 (TRPV1), a polymodal nociceptive receptor, in the ear (11). These results suggest that neurogenic inflammation via TRPV1 and neurotrophins may play a key role in FA-induced dermatitis.

TRPV1 is activated by a variety of stimuli. Heat (> 43°C) (12, 13), low pH (12, 13), and chemicals including capsaicin (12, 13), camphor (14), nitric oxide (15), and spider toxins (16) are known as exogenous activators of TRPV1. TRPV1 is expressed in nociceptive primary afferent neurons (12, 17) and TRPV1-positive neurons colocalize with neuropeptides such as substance P and calcitonin gene-related peptide (18); therefore, activation of TRPV1 can trigger neurogenic inflammation via neuropeptide secretion from neurons. In fact, capsaicin, a TRPV1 ligand, induces neuropeptides secretion from dorsal root ganglion neurons in vitro (19, 20). In addition, several studies have implied a relationship between TRPV1 and pain sensation or inflammation in vivo. Davis et al. demonstrated that TRPV1 is essential for thermal hyperalgesia (21). On the other hand, silencer RNA for TRPV1 suppressed cold hypersensitivity induced by ligation of sciatic nerve in rats (22). Similarly, TRPV1 antisense oligodeoxynucleotides reduced mechanical hyperalgesia in rats caused by spinal nerve ligation (23). Furthermore, TRPV1 is reported to be activated by bradykinin, an inflammatory mediator (24), and nociceptive behavior induced by bradykinin was reduced in TRPV1 gene–knockout (TRPV1 KO) mice compared to wild-type (WT) mice (25). However, the involvement and functional relevance of TRPV1 in FA-induced skin inflammation is still unknown.

In the present study, we examined whether TRPV1 is involved in skin inflammation induced by repeated FA application to mouse ears. To evaluate the functional relevance of TRPV1 in FA-induced skin inflammation, we compared ear swelling caused by FA in TRPV1 KO mice to that in WT mice and examined the effect of a threshold dose of capsaicin, a TRPV1 agonist, on ear skin response after repetitive painting with FA.

Materials and Methods

Animals

Female BALB/c mice (Japan SLC, Inc., Hamamatsu), C57BL/6 mice (Japan SLC, Inc.), and TRPV1 KO mice (C57BL/6 mouse background) were used. All animals were housed in plastic cages in an air-conditioned room at 22°C ± 1°C with relative humidity of 60% ± 5%, fed a standard laboratory diet, and given water ad libitum. All experiments were carried out following the rules and regulations for the care and use of experimental animals established by Gifu Pharmaceutical University in 2008.

Reagents

The following drugs and chemicals were purchased commercially: FA solution (37%) (Kishida Chemical Co., Ltd., Osaka); ISOGEN (Nippon Gene, Toyama); acetone and diethyl pyrocarbonate-treated water (DEPC water) (Nacalai Tesque, Inc.); random primer, 5 × first-strand buffer, 0.1 M dithiothreitol (DTT), and SuperScript RNase H-reverse transcriptase (Invitrogen, Carlsbad, CA, USA); PCR primers [β-actin, brain-derived neurotrophic factor (BDNF), interferon-γ (IFN-γ), IL-4, nerve growth factor (NGF), neurotrophin-3 (NT-3), glial cell–derived neurotrophic factor (GDNF), TRPV1] and capsaicin (Sigma-Aldrich Japan Co., Ltd., Tokyo); 10 × PCR buffer, 10 mM deoxynucleotide triphosphate (dNTP), and SYBR GREEN (Takara Bio, Inc., Shiga).

Ear swelling response induced by topical FA application

FA was dissolved in acetone and 1% and 5% FA solutions were prepared. Twenty-five microliters of vehicle (acetone) or FA solution were applied to dorsal and ventral surfaces of both ear lobes once a week for 5 weeks. Cutaneous reaction was evaluated by measuring the thickness of ear lobes. Ear thickness was measured immediately before and at 1, 4, and 24 h after each application of FA solution using a micrometer (Peacock Upright Dial Gauge; Ozaki MFG Co., Tokyo). Results are expressed as increased ear thickness after subtracting the value obtained before the first FA application from each value.

Histopathological study of ear lesions

Mouse ears were excised 24 h after the fifth painting with vehicle or 5% FA solution and fixed with 10% buffered formalin. Ear segments were cut into parasagittal slices, dehydrated, and embedded in paraffin by standard procedures. Paraffin sections were stained with hematoxylin and eosin or toluidine blue and assessed by light microscopy.

RNA extraction and cDNA synthesis

Mice were sacrificed 24 h after the fifth vehicle or 1% or 5% FA application. The ears were excised and quickly frozen with liquid N_2. The ear samples were homogenized in 1 mL ISOGEN using a POLYTRON (Kinematica AG, Lucerne, Switzerland). The homogenate was mixed vigorously with 0.2 mL chloroform and centrifuged at 12,000 × g for 15 min at 4°C. The supernatant was col-

lected and 0.5 mL isopropanol was added. The mixture was centrifuged at 12,000 × *g* for 10 min at 4°C, and then the RNA precipitate was washed once with 1 mL of 75% ethanol and dissolved in 0.1 mL DEPC water. The concentration of total RNA in each sample was measured at 260 nm with Gene Quant (Pharmacia Biotech, Cambridge, UK).

Complementary DNA was synthesized using the following procedures: 12 μL of reaction mixtures containing 1 μg RNA and random primer were incubated for 10 min at 70°C and chilled on ice immediately. Four microliters of 5 × first-strand buffer, 1 μL of 10 mM dNTP, and 2 μL of 0.1 M DTT were added to the mixture and incubated for 5 min at room temperature. SuperScript RNase H-reverse transcriptase was added; and the samples were incubated for 10 min at 25°C, for 50 min at 42°C, and for 15 min at 70°C sequentially and then quickly chilled on ice.

Analysis of neurotrophins and TRPV1 mRNA expressions in ears by real-time RT-PCR

Real-time reverse transcriptase–polymerase chain reaction (RT-PCR) was performed with an iCycler Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). The following components were added to the cDNA (1 μg): 9 μL DEPC water, 1.25 μL sense primer, 1.25 μL antisense primer, and 12.5 μL SYBR GREEN. The sequences of primers used in the present study are listed in Table 1.

Real-time RT-PCR procedures were as follows: samples were denatured with 94°C for 5 s and annealed with primers and extended with 62°C for 30 s. These reactions were repeated for 40 cycles. The mRNA expressions of various molecules were normalized with β-actin mRNA expression.

Table 1. Primer sequences used for RT-PCR analysis

cDNA	Primer sequences (5′ to 3′)	
β-Actin	Forward:	5′-GATCTGGCACCACACCTTCT-3′
	Reverse:	5′-GGGGTGTTGAAGGTCTCAAA-3′
BDNF	Forward:	5′-CAGTGACAGGCGTTGAGAAA-3′
	Reverse:	5′-AACGCCCTCATTCTGAGAGA-3′
GDNF	Forward:	5′-GCCCAGCTACAGAAAACTGG-3′
	Reverse:	5′-ACTGGCTTGGTTCTTTGCAT-3′
IFN-γ	Forward:	5′-GAGGAACTGGCAAAAGGATG-3′
	Reverse:	5′-GCTGATGGCCTGATTGTCTT-3′
IL-4	Forward:	5′-ACAGGAGAAGGGACGCCAT-3′
	Reverse:	5′-GAAGCCCTACAGACGAGCTCA-3′
NGF	Forward:	5′-AATAGCTGCCCGATGGACAG-3′
	Reverse:	5′-GTCTGAAGAGGTGGGTGGAG-3′
NT-3	Forward:	5′-TGCAACGGACACAGAGCTAC-3′
	Reverse:	5′-TGCCCACATAATCCTCCATT-3′
TRPV-1	Forward:	5′-TGTGGAGGTGGCAGATAACA-3′
	Reverse:	5′-CTTCAGTGTGGGGTGGAGTT-3′

Ear swelling caused by capsaicin

Twenty-five microliters of vehicle (acetone) or 5% FA solution were applied to dorsal and ventral surfaces of both ear lobes of BALB/c mice once a week for 5 weeks. Six days after the final FA painting, 10 μg capsaicin was applied to the ears of FA-treated or vehicle-treated mice. Ear thickness was measured immediately before and at 0.5, 1.5, 2, 4, and 24 h after each application of capsaicin solution using a micrometer. Results are expressed as increased ear thickness after subtracting the value obtained before the first FA application from each value.

Statistical analyses

Results are represented as the mean ± S.E.M. Statistical significance between two groups was estimated using the two-tailed Student's *t*-test or Mann Whitney's *U*-test after data variances had been evaluated by the *F*-test. To define statistically significant differences among three groups of mice, the data were examined by a nonparametric test or parametric Dunnett's multiple comparison test after confirming the data variance using Bartlett's test. $P < 0.05$ was considered significant.

Results

Effect of repeated painting of FA on the expression of various mRNAs in BALB/c mice

In our previous study, we demonstrated that repeated FA application at concentrations of 2% – 10% to mouse ears increased mRNA expressions of IL-4, neurotrophins, and TRPV1 using pooled samples in each group by RT-PCR (11). In the present study, to confirm the results of the previous study, we first reanalyzed mRNA expression in ears exposed to FA repeatedly using real-time RT-PCR analyses.

FA solution was painted onto mouse ears once a week for 5 weeks, and the ears were excised 24 h after the final FA application and analyzed for various mRNA expressions. Marked ear swelling was induced by the second and third FA painting and the response peaked 1 h after the FA application; however, at the fourth and fifth painting, biphasic ear swelling was observed with peak responses 1 and 24 h after FA application, as described previously (11) (data not shown). As shown in Fig. 1, IL-4 and IFN-γ mRNA levels were increased by 5% FA painting, whereas vehicle and 1% FA application did not affect mRNA expression of these cytokines. In addition, NGF, BDNF GDNF, NT-3, and TRPV1 mRNA levels were significantly increased by FA application in a con-

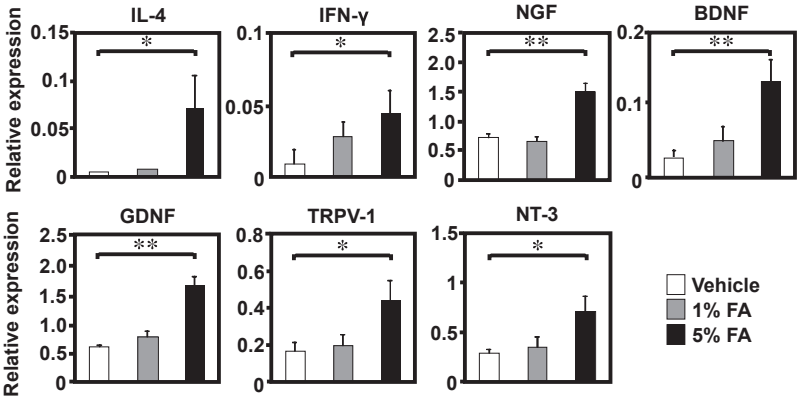

Fig. 1. mRNA expression of IFN-γ, IL-4, neurotrophins, and TRPV1 in ears of BALB/c mice exposed repeatedly to FA. Ears were excised 24 h after the 5th painting. The mRNA expressions were analyzed by real-time RT-PCR. Data are presented as the means ± S.E.M. of 5 – 7 mice. *$P < 0.05$, **$P < 0.01$ (vs. vehicle).

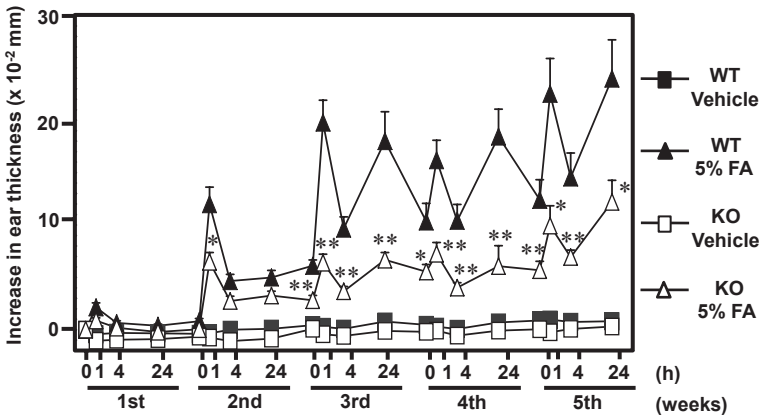

Fig. 2. Time-course study of ear swelling caused by repeated painting with FA in TRPV1 KO mice and WT mice (C57BL/6 mice). FA was painted 5 times onto dorsal and ventral surfaces of both ears, and ear thickness was measured at 0, 1, 4, and 24 h after each painting. Data are presented as the means ± S.E.M. of 5 – 7 mice. *$P < 0.05$, **$P < 0.01$ (vs. WT 5% FA).

centration-dependent fashion. These results suggest that immunological and neurogenic mechanisms are involved in FA-induced skin inflammation.

FA-induced ear swelling response in TRPV1 KO mice

To investigate the functional significance of TRPV1 in FA-induced dermatitis, we examined the ear-swelling response caused by repeated FA painting in TRPV1 KO mice. The ear-swelling response induced by second and third FA painting peaked 1 h after FA painting in WT mice. In addition, fourth and fifth FA painting provoked biphasic ear-swelling responses that peaked 1 and 24 h, respectively, after painting in WT mice. In contrast, the magnitude of the ear-swelling response caused by second to fifth FA painting was significantly attenuated in TRPV1 KO mice compared to WT mice (Fig. 2).

Histopathological analysis of ears applied with FA repeatedly

Figure 3 shows representative sections of mouse ears of TRPV1 KO and the corresponding WT mice exposed to 5% FA solution. Mouse ears were excised 24 h after the fifth painting and the sections were stained with hematoxylin and eosin or toluidine blue. Infiltration of inflammatory cells such as neutrophils, eosinophils, and monocytes and hypertrophy of the epidermis were evident (WT 5% FA), and some mast cell invasion into the dermis (arrows in WT 5% FA) was observed in the ears of WT mice exposed to FA. In contrast, these histological changes were milder in the ears of TRPV1 KO mice exposed to FA than in WT mice.

Effect of repeated painting of FA on the expression of various mRNAs in TRPV1 KO and WT mice

Next, we analyzed mRNA expressions in the ear 24 h after final FA painting by real-time RT-PCR. IL-4 and IFN-γ mRNA levels were increased in the ears of WT and TRPV1 KO mice painted with 5% FA repeatedly. There were no differences in the expression levels of IL-4 and IFN-γ mRNA between WT and TRPV1 KO mice. On the other hand, NGF, BDNF, GDNF, and NT-3 mRNA expression levels were significantly decreased in FA-treated TRPV1 KO mice compared to FA-treated WT mice (Fig. 4).

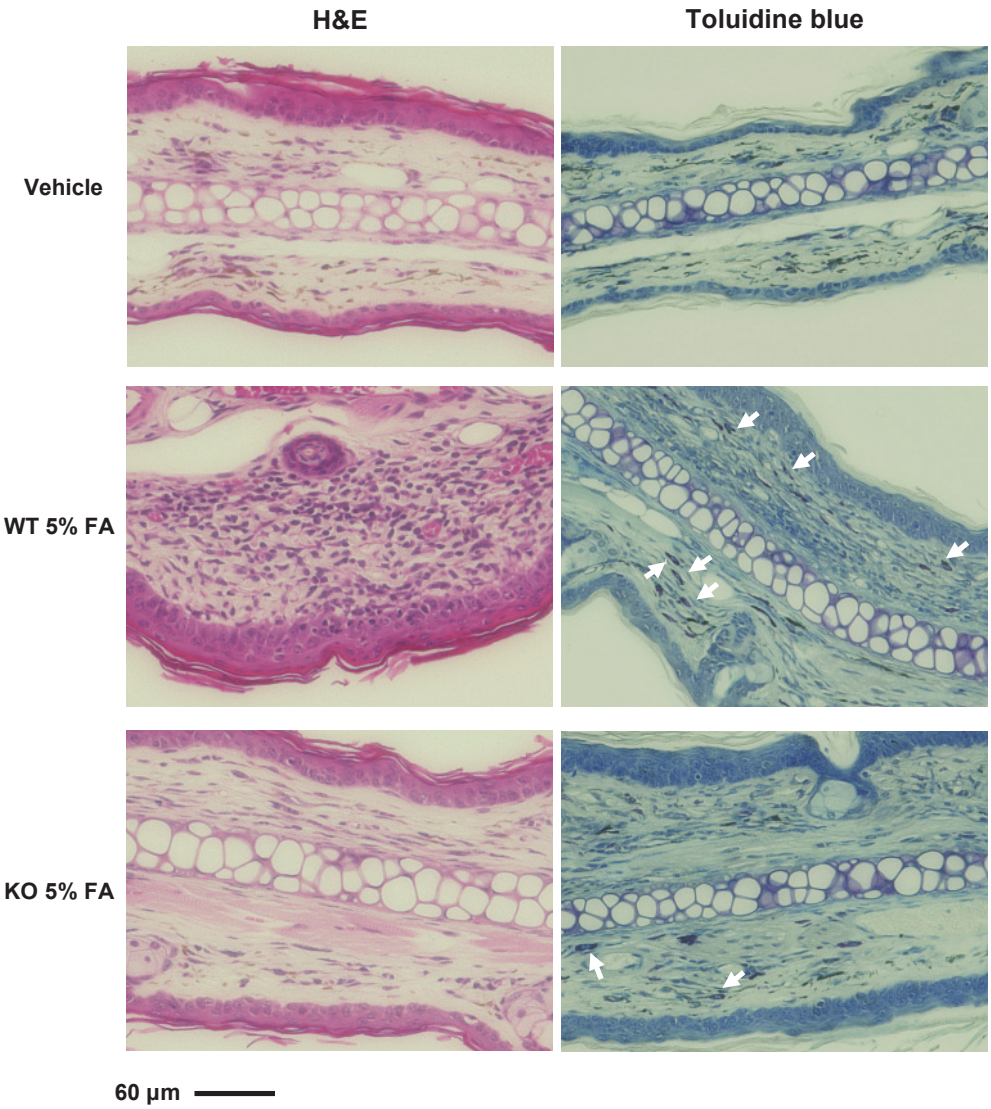

Fig. 3. Histological changes of ears in TRPV1 KO and WT mice (C57BL/6 mice) exposed to 5% FA. Mouse ears were excised 24 h after the 5th painting with 5% FA. Each section was stained with hematoxylin and eosin or toluidine blue. Arrows show mast cells that were increased in 5% FA–treated wild-type mice compared to vehicle-treated mice or to 5% FA–treated TRPV1 gene–knockout mice.

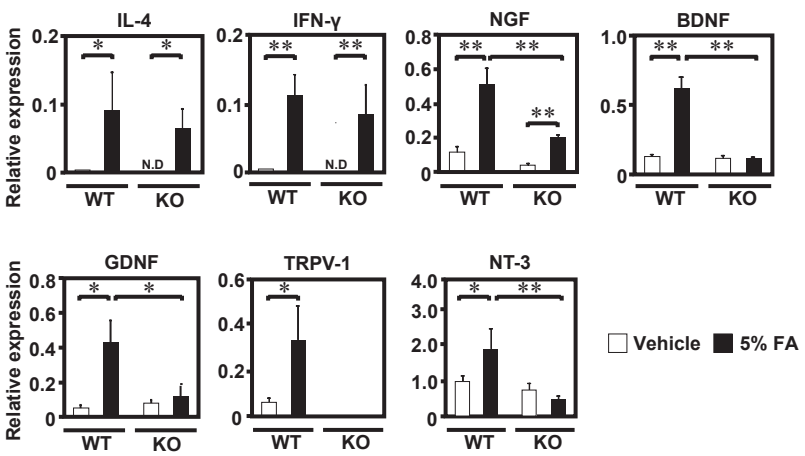

Fig. 4. mRNA expression of IFN-γ, IL-4, neurotrophins, and TRPV1 in ears in TRPV1 KO and WT mice (C57BL/6 mice) exposed to 5% FA. Ears were excised 24 h after the 5th painting. The mRNA expressions were analyzed by real-time RT-PCR. Data are presented as the means ± S.E.M. of 5 – 7 mice. *$P < 0.05$, **$P < 0.01$.

Ear-swelling response to capsaicin, a TRPV1 agonist, after repeated FA painting

To investigate whether TRPV1 is functionally expressed in inflamed skin by repeated FA provocation, we evaluated ear swelling by capsaicin painting six days after the fifth FA application when ear thickness had recovered to the control level. Mice were painted with a threshold dose of capsaicin (10 μg), with which ear swelling was not observed in vehicle-treated mice (Fig. 5). As depicted in Fig. 5, marked ear swelling was observed in FA-treated mice, and the response peaked 30 min after capsaicin painting and had completely attenuated by 60 min after painting. These results indicate that repetitive FA application to mouse ears induces a functional expression of TRPV1.

Discussion

In the present study, we investigated the mechanism of skin inflammation caused by repeated painting of 1% or 5% FA onto mouse ears. Exposure to 5% of FA increased BDNF, GDNF, IFN-γ, IL-4, NGF, NT-3, and TRPV1 mRNAs in the ears of BALB/c mice. Furthermore, the ear-swelling response and increased neurotrophins mRNA levels in 5% FA treated group were significantly attenuated in TRPV1 KO mice compared with those in WT mice. These results demonstrate that TRPV1 is involved in ear swelling induced by FA exposure and suggest that TRPV1 contributes to the induction of FA-induced neurogenic inflammation possibly through the production of neurotrophins.

Increased IFN-γ and IL-4 mRNA levels in the ears by FA application implies that FA acts as a hapten to induce dermatitis. Cumulative evidence shows that dinitrofluorobenzene (DNFB) and fluorescein isothiocyanate (FITC), representative haptens, increase the production of IFN-γ or IL-4 in a murine model of contact hypersensitivity (CHS) or atopic dermatitis (26 – 28). Although CHS induced by DNFB results in the Th1 dominant immune response, repeated DNFB application increases expression of IL-4, a representative Th2 cytokine (28). Therefore, it is reasonable that both IFN-γ and IL-4 mRNA expression was elevated by FA painting regardless of whether FA is a Th1 or Th2 dominant hapten. In the present study, we have not yet investigated the role of these cytokines in the onset of FA-induced ear swelling; however, FA seems to induce skin inflammation via IL-4 production because we found that the ear-swelling response induced by repeated painting of 5% FA was diminished in IL-4 KO mice compared to WT mice (unpublished data). Since IL-4 is critical for the production of IgE, we measured serum IgE level during the first to 5th FA painting. Intriguingly, and contrary to our expectation, FA-specific IgE was not detected at all (data not shown) (11), suggesting that IgE is not involved in FA-induced skin inflammation. On the other hand, there were no differences in IFN-γ and IL-4 mRNA levels between TRPV1 KO mice and WT mice after FA painting on the ear, indicating that TRPV1 may not affect the production of these cytokines in FA-induced skin inflammation in mice.

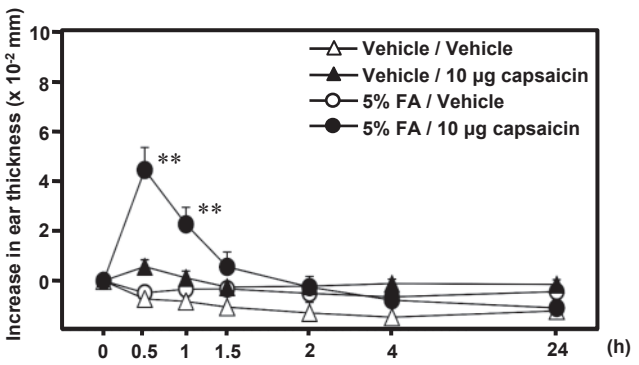

Fig. 5. Ear swelling response to capsaicin, a TRPV1 agonist, after repeated FA painting. Mice were painted with acetone (vehicle) or 5% FA, once a week, 5 times. Capsaicin (10 μg) was applied to mouse ears 6 days after the 5th painting, and ear thickness was measured immediately prior to capsaicin painting and at 0.5, 1, 1.5, 2, 4, and 24 h after capsaicin painting. Results are the means ± S.E.M. of 5 – 7 mice. $^{**}P < 0.01$ (vs. "vehicle / 10 μg capsaicin" group).

In the present study, we demonstrated that TRPV1 plays a crucial role in FA-induced skin inflammation. Recently, some reports described an important role of TRPV1 in hapten-induced CHS. Interestingly, TRPV1 has a protective role in DNFB-induced CHS by supporting the function of palmitoylethanolamide, an endogenous anti-inflammatory factor (29). Additionally, oxazolone-induced CHS is diminished in TRPV1 KO mice compared with WT mice (30). Yet, the role of TRPV1 in FITC-induced CHS is still unclear. However, dibutyl phthalate, which is used as the solvent and adjuvant of FITC, activates TRPV1 directly and is involved in the development of inflammation (31). The results reported in these studies suggest that the role of TRPV1 in CHS is dependent on the characteristics of the hapten.

Neurotrophins not only control nerve growth, survival, maintenance, and differentiation, but several reports have demonstrated that neurotrophins also contribute to hyperalgesia or inflammation. Some studies demonstrate that, NGF can induce thermal and mechanical hyperalgesia in rats (32 – 34). In addition, BDNF, NT-3, and GDNF are responsible for the development of hyperalgesia or promote hyperalgesia in rodents (35 – 38). Furthermore, NGF enhances TRPV1 signal transduction and promotes translocation of TRPV1 to the cell surface via PI3K or

PKC (39, 40), suggesting that neurotrophins contribute to pain or inflammation by enhancing the activation or sensitivity of TRPV1. Although the interaction between TRPV1 and neurotrophins has not been fully investigated except for NGF, we speculate that neurotrophins other than NGF are also involved in the development or exacerbation of FA-induced skin inflammation by enhancing the activation or sensitivity of TRPV1. In fact, as shown in Fig. 4, TRPV1-depletion by gene-knockout decreased mRNA levels of neurotrophins in FA-treated mice, implying that TRPV1 and neurotrophins interact with each other when nociceptive stimuli invaded skin. In addition, human keratinocytes that produce NGF express TRPV1 (41, 42). Therefore, activation of TRPV1 might lead to NGF secretion from keratinocytes. In chronic skin inflammation such as our FA-induced dermatitis model, neurotrophins including NGF derived from keratinocytes could promote nerve growth and production of neuropeptides that provoke edema. In fact, we found decreased ear swelling in TRPV1 KO mice. Taken together, neurotrophins might be involved in the development of skin inflammation induced by FA; therefore, further experiments are needed to clarify the types of cell that produce neurotrophins in this model and their functional relevance.

In addition to neurotrophins, TRPV1 mRNA was upregulated by repeated FA painting. Moreover, the ear-swelling response was significantly attenuated in TRPV1 KO mice compared to WT mice. TRPV1 is activated by various nociceptive stimuli, such as acid (proton) (13), heat (> 43°C) (12), and capsaicin, an irritant derived from chili pepper (12) suggested to promote neurogenic inflammation via neuropeptide release, which increases vascular permeability and causes edema (43, 44). Therefore, in our mouse model of skin inflammation, FA could activate TRPV1 directly or indirectly by inflammatory mediators such as bradykinin, prostaglandin E_2, arachidonic acid, and neurotrophins known to activate TRPV1 indirectly via PKA or PKC (45 – 47) to release neuropeptides and cause skin inflammation. In fact, our previous study demonstrated that FA-induced ear swelling was inhibited by treatment with capsazepine, a TRPV1 antagonist (11). Likewise, in the present study, a threshold dose of capsaicin in which ear swelling was not observed in vehicle-treated mice caused marked ear swelling in FA-treated mice. These findings demonstrate that repeated stimulation with FA increases TRPV1 expression in inflamed tissue and that the activation of TRPV1 through inflammatory mediators and neurotrophins is therefore one of the possible mechanisms of FA-induced skin inflammation. The cell or nerve types expressing TRPV1 after FA application should be further examined.

TRPV1-positive sensory nerves co-express another TRP channel, TRPA1 (48, 49). TRPA1 is activated by cold temperature below 18°C (48, 50), as well as by mustard oil and cinnamon oil (51, 52). Since TRPA1 KO mice are less responsive to noxious mechanical stimuli than WT mice (53), TRPA1 is necessary to sense mechanical stimuli. TRPA1, as well as TRPV1, is activated indirectly by bradykinin through G-protein-coupled bradykinin receptor 2 and PLC in vitro (50) and bradykinin-induced mechanical hyperalgesia is milder in TRPA1 KO mice than in WT mice (53). It is noteworthy that FA directly activates TRPA1 in HEK293 cells expressing TRPA1 (54) and the response to FA injection is reduced in TRPA1- KO mice compared to WT mice (54). Taken together, TRPA1 might be a candidate molecule contributing to the ear-swelling response caused by repeated painting with FA; therefore, further experiments are needed to clarify whether TRPA1 is involved in this model.

In conclusion, we demonstrated that TRPV1 plays a pivotal role in FA-induced skin inflammation in mice and that TRPV1 may be involved in home-related symptoms caused by FA.

Acknowledgments

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References

1 Finnegan MJ, Pickering CA, Burge PS. The sick building syndrome: prevalence studies. Br Med J (Clin Res Ed). 1984;289: 1573–1575.
2 World Health Organization. Indoor air pollutants; exposure and health effects assessment. Working Group Report, Nordingen, Euro Reports and Studies No. 78. 1983.
3 Fang L, Wyon DP, Clausen G, Fanger PO. Impact of indoor air temperature and humidity in an office on perceived air quality, SBS symptoms and performance. Indoor Air. 2004;14:74–81.
4 Stark PC, Burge HA, Ryan LM, Milton DK, Gold DR. Fungal levels in the home and lower respiratory tract illnesses in the first year of life. Am J Respir Crit Care Med. 2003;168:232–237.
5 Saijo Y, Kishi R, Sata F, Katakura Y, Urashima Y, Hatakeyama A, et al. Symptoms in relation to chemicals and dampness in newly built dwellings. Int Arch Occup Environ Health. 2004;77: 461–470.
6 Leikauf GD. Mechanisms of aldehyde-induced bronchial reactivity: role of airway epithelium. Res Rep Health Eff Inst. 1992: 1–35.
7 World Health Organization. Environmental health criteria No. 89. Formaldehyde. 1997.
8 Barnes PJ. Neuroeffector mechanisms: the interface between in-

flammation and neuronal responses. J Allergy Clin Immunol. 1996;98:S73–S81.
9 Ito K, Sakamoto T, Hayashi Y, Morishita M, Shibata E, Sakai K, et al. Role of tachykinin and bradykinin receptors and mast cells in gaseous formaldehyde-induced airway microvascular leakage in rats. Eur J Pharmacol. 1996;307:291–298.
10 Meggs WJ. Mechanisms of allergy and chemical sensitivity. Toxicol Ind Health. 1999;15:331–338.
11 Saito A, Tanaka H, Usuda H, Shibata T, Higashi S, Yamashita H, et al. Characterization of skin inflammation induced by repeated exposure of toluene, xylene, and formaldehyde in mice. Environ Toxicol. 2011;26:224–232.
12 Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature. 1997;389:816–824.
13 Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, et al. The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron. 1998;21:531–543.
14 Xu H, Blair NT, Clapham DE. Camphor activates and strongly desensitizes the transient receptor potential vanilloid subtype 1 channel in a vanilloid-independent mechanism. J Neurosci. 2005;25:8924–8937.
15 Yoshida T, Inoue R, Morii T, Takahashi N, Yamamoto S, Hara Y, et al. Nitric oxide activates TRP channels by cysteine S-nitrosylation. Nat Chem Biol. 2006;2:596–607.
16 Siemens J, Zhou S, Piskorowski R, Nikai T, Lumpkin EA, Basbaum AI, et al. Spider toxins activate the capsaicin receptor to produce inflammatory pain. Nature. 2006;444:208–212.
17 Sanchez JF, Krause JE, Cortright DN. The distribution and regulation of vanilloid receptor VR1 and VR1 5′ splice variant RNA expression in rat. Neuroscience. 2001;107:373–381.
18 Bae YC, Oh JM, Hwang SJ, Shigenaga Y, Valtschanoff JG. Expression of vanilloid receptor TRPV1 in the rat trigeminal sensory nuclei. J Comp Neurol. 2004;478:62–71.
19 Anand U, Otto WR, Casula MA, Day NC, Davis JB, Bountra C, et al. The effect of neurotrophic factors on morphology, TRPV1 expression and capsaicin responses of cultured human DRG sensory neurons. Neurosci Lett. 2006;399:51–56.
20 Yang X, Gong H, Liu Z, Liu H, Wang H, Li Z. Similar and different effects of capsaicin and resiniferatoxin on substance P release and transient receptor potential vanilloid type 1 expression of cultured rat dorsal root ganglion neurons. Methods Find Exp Clin Pharmacol. 2010;32:3–11.
21 Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P, et al. Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. Nature. 2000;405:183–187.
22 Christoph T, Grunweller A, Mika J, Schäfer MK, Wade EJ, Weihe E, et al. Silencing of vanilloid receptor TRPV1 by RNAi reduces neuropathic and visceral pain in vivo. Biochem Biophys Res Commun. 2006;350:238–243.
23 Christoph T, Gillen C, Mika J, Grünweller A, Schäfer MK, Schiene K, et al. Antinociceptive effect of antisense oligonucleotides against the vanilloid receptor VR1/TRPV1. Neurochem Int. 2007;50:281–290.
24 Shin J, Cho H, Hwang SW, Jung J, Shin CY, Lee SY, et al. Bradykinin-12-lipoxygenase-VR1 signaling pathway for inflammatory hyperalgesia. Proc Natl Acad Sci U S A. 2002;99:10150–10155.
25 Katanosaka K, Banik RK, Giron R, Higashi T, Tominaga M, Mizumura K. Contribution of TRPV1 to the bradykinin-evoked nociceptive behavior and excitation of cutaneous sensory neurons. Neurosci Res. 2008;62:168–175.
26 Takeshita K, Yamasaki T, Akira S, Gantner F, Bacon KB. Essential role of MHC II-independent $CD4^+$ T cells, IL-4 and STAT6 in contact hypersensitivity induced by fluorescein isothiocyanate in the mouse. Int Immunol. 2004;16:685–695.
27 Nagai H, Ueda Y, Tanaka H, Hirano Y, Nakamura N, Inagaki N, et al. Effect of overproduction of interleukin 5 on dinitrofluorobenzene-induced allergic cutaneous response in mice. J Pharmacol Exp Ther. 1999;288:43–50.
28 Inagaki N, Shiraishi N, Igeta K, Itoh T, Chikumoto T, Nagao M, et al. Inhibition of scratching behavior associated with allergic dermatitis in mice by tacrolimus, but not by dexamethasone. Eur J Pharmacol. 2006;546:189–196.
29 Petrosino S, Cristino L, Karsak M, Gaffal E, Ueda N, Tüting T, et al. Protective role of palmitoylethanolamide in contact allergic dermatitis. Allergy. 2010;65:698–711.
30 Bánvölgyi A, Pálinkás L, Berki T, Clark N, Grant AD, Helyes Z, et al. Evidence for a novel protective role of the vanilloid TRPV1 receptor in a cutaneous contact allergic dermatitis model. J Neuroimmunol. 2005;169:86–96.
31 Shiba T, Maruyama T, Kurohane K, Iwasaki Y, Watanabe T, Imai Y. TRPA1 and TRPV1 activation is a novel adjuvant effect mechanism in contact hypersensitivity. J Neuroimmunol. 2009;207:66–74.
32 Lewin GR, Ritter AM, Mendell LM. Nerve growth factor-induced hyperalgesia in the neonatal and adult rat. J Neurosci. 1993;13:2136–2148.
33 Lewin GR, Rueff A, Mendell LM. Peripheral and central mechanisms of NGF-induced hyperalgesia. Eur J Neurosci. 1994;6:1903–1912.
34 Shu XQ, Llinas A, Mendell LM. Effects of trkB and trkC neurotrophin receptor agonists on thermal nociception: a behavioral and electrophysiological study. Pain. 1999;80:463–470.
35 Groth R, Aanonsen L. Spinal brain-derived neurotrophic factor (BDNF) produces hyperalgesia in normal mice while antisense directed against either BDNF or trkB, prevent inflammation-induced hyperalgesia. Pain. 2002;100:171–181.
36 Li CQ, Xu JM, Liu D, Zhang JY, Dai RP. Brain derived neurotrophic factor (BDNF) contributes to the pain hypersensitivity following surgical incision in the rats. Mol Pain. 2008;4:27.
37 White DM. Neurotrophin-3 antisense oligonucleotide attenuates nerve injury-induced Abeta-fibre sprouting. Brain Res. 2000;885:79–86.
38 Malin SA, Molliver DC, Koerber HR, Cornuet P, Frye R, Albers KM, et al. Glial cell line-derived neurotrophic factor family members sensitize nociceptors in vitro and produce thermal hyperalgesia in vivo. J Neurosci. 2006;26:8588–8599.
39 Stein AT, Ufret-Vincenty CA, Hua L, Santana LF, Gordon SE. Phosphoinositide 3-kinase binds to TRPV1 and mediates NGF-stimulated TRPV1 trafficking to the plasma membrane. J Gen Physiol. 2006;128:509–522.
40 Zhu W, Oxford GS. Phosphoinositide-3-kinase and mitogen activated protein kinase signaling pathways mediate acute NGF sensitization of TRPV1. Mol Cell Neurosci. 2007;34:689–700.
41 Denda M, Fuziwara S, Inoue K, Denda S, Akamatsu H, Tomitaka A, et al. Immunoreactivity of VR1 on epidermal keratinocyte of human skin. Biochem Biophys Res Commun. 2001;285:1250–1252.
42 Southall MD, Li T, Gharibova LS, Pei Y, Nicol GD, Travers JB.

Activation of epidermal vanilloid receptor-1 induces release of proinflammatory mediators in human keratinocytes. J Pharmacol Exp Ther. 2003;304:217–222.

43 Lee LY, Gu Q. Role of TRPV1 in inflammation-induced airway hypersensitivity. Curr Opin Pharmacol. 2009;9:243–249.

44 Alawi K, Keeble J. The paradoxical role of the transient receptor potential vanilloid 1 receptor in inflammation. Pharmacol Ther. 2010;125:181–195.

45 Calixto JB, Kassuya CA, Andre E, Ferreira J. Contribution of natural products to the discovery of the transient receptor potential (TRP) channels family and their functions. Pharmacol Ther. 2005;106:179–208.

46 Tominaga M, Tominaga T. Structure and function of TRPV1. Pflugers Arch. 2005;451:143–150.

47 Morenilla-Palao C, Planells-Cases R, Garcia-Sanz N, Ferrer-Montiel A. Regulated exocytosis contributes to protein kinase C potentiation of vanilloid receptor activity. J Biol Chem. 2004; 279:25665–25672.

48 Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, Hricik TR, et al. ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell. 2003; 112:819–829.

49 Kobayashi K, Fukuoka T, Obata K, Yamanaka H, Dai Y, Tokunaga A, et al. Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with adelta/c-fibers and colocalization with trk receptors. J Comp Neurol. 2005;493: 596–606.

50 Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, et al. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron. 2004;41:849–857.

51 Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Hogestatt ED, et al. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature. 2004;427:260–265.

52 Macpherson LJ, Geierstanger BH, Viswanath V, Bandell M, Eid SR, Hwang S, et al. The pungency of garlic: activation of TRPA1 and TRPV1 in response to allicin. Curr Biol. 2005;15:929–934.

53 Kwan KY, Allchorne AJ, Vollrath MA, Christensen AP, Zhang DS, Woolf CJ, et al. TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. Neuron. 2006;50:277–289.

54 McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, Zhao M, et al. TRPA1 mediates formalin-induced pain. Proc Natl Acad Sci U S A. 2007;104:13525–13530.