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Type 2 cytokine-JAK1 signaling is involved in the development of dry-skin induced mechanical alloknesis

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ABBREVIATIONS: AD, atopic dermatitis; AEW, acetone-ether and water; IL, interleukin; ILC2, group 2 innate lymphoid cells; JAK, Janus kinase; m-alloknesis, mechanical alloknesis; NT, non-treated; SC, stratum corneum; STAT, signal transducer and activator of transcription; TEWL, transepidermal water loss; Th2, type 2 T helper cells; TSLP, thymic stromal lymphopoietin; W, water.

ABSTRACT

Background: Mechanical alloknesis (m-alloknesis) is itch hypersensitivity induced by normally innocuous stimuli. It is sometimes observed in dry skin based itch-related diseases such as atopic dermatitis (AD), and often triggers the vicious itch-scratch cycle. The acetone-ether and water (AEW) mouse model mimics dry skin induced m-alloknesis, yet its underlying mechanism remains unclear. Janus kinase (JAK) inhibitors are used to treat AD, but their effects on m-alloknesis are not fully known.

Objective: To reveal the effects of various oral JAK inhibitors on m-alloknesis and their action points, using AEW model.

Methods: AEW model was prepared by treatment with a mixture of acetone-ether, and they were orally administrated a JAK1/2 inhibitor baricitinib, a selective JAK1 inhibitor abrocitinib, or a JAK2 selective inhibitor AZ960, and evaluated m-alloknesis score as the total number of scratching responses in 30 mechanical stimulations. To further elucidate the mechanism of action, IL-4, IL-13 or thymic stromal lymphopoietin (TSLP) or their neutralizing antibodies were also applied to mice. In addition, the levels of these cytokines in mouse skin were measured using multiple immunoassays.

Results: All of JAK inhibitors effectively reduced m-alloknesis, with abrocitinib demonstrating the most significant inhibition. The neutralizing antibodies against IL-4,

IL-13, and TSLP inhibited m-alloknesis in AEW mice. Intradermal administration of IL-4, IL-13, or TSLP induced m-alloknesis, and abrocitinib effectively mitigated each cytokine-induced response. Highly sensitive assays detected IL-4, IL-13, IL-31 and TSLP in AEW-treated skin, with TSLP levels significantly increased.

Conclusion: Type 2 cytokine-JAK1 signaling is involved in the development of m-alloknesis in dry skin.

Key words: atopic dermatitis, dry skin, JAK inhibitors, mechanical alloknesis, mechanical itch, Th2 cytokines

1. Introduction

When skin barrier function is impaired due to environmental factors such as temperature, and humidity, or genetic factors such as filaggrin mutations, the skin loses water and becomes dry and itchy [1, 2] This manifestation, known as dry skin, is a common feature of dermatological diseases like atopic dermatitis (AD) and systemic conditions associated with itch, such as diabetes mellitus and chronic kidney disease [2, 3]. To investigate itch induction factors, the efficacy of antipruritic drugs and their mechanisms of action, researchers have adopted the AEW (acetone-ether and water)

mouse model of dry skin [3-5]. This model also mimics mechanical alloknesis (m-alloknesis), an itch hypersensitivity phenomenon induced by normally innocuous mechanical stimuli [6], although the underlying mechanisms remain unclear. Janus kinase (JAK) inhibitors, including baricitinib and abrocitinib, have recently gained attention as therapeutic agents for AD due to their rapid and significant effects on itch [7, 8]. However, their effects on m-alloknesis remain unclear. This study aimed to assess the effectiveness and action points of various JAK inhibitors on m-alloknesis using AEW dry-skin model mouse.

2. Methods

2.1. Animals

Male C57BL/6J mice obtained from Oriental Yeast Co., Ltd. (Tokyo, Japan) at age 6 to 7 weeks old were subsequently kept in-house and used in the experiments at 8-weeks old. They were maintained under controlled conditions of temperature (range, 23-25°C) and light (8:00 AM to 8:00 PM) with food and water provided *ad libitum*. All animal experiments in this study were approved by Juntendo University Animal Ethics Committee, authorization numbers: 2021102, 2022068, and 2023106.

2.2. Preparation of dry skin model

The rostral back of each mouse was shaved at least 2-days prior to treatment. A mixture of 1:1 ratio acetone and diethyl ether was applied to the shaved area for 15 s, followed by distilled water for 30 s. This process was repeated twice a day for 6-days to establish the dry skin model group (AEW-treated mice). Two similarly shaved control groups were included: W-treated mice received only distilled water for 45 s; (W-treated mice), and the other had their rostral backs shaved without further treatment (not-treated: NT mice). These control groups served as negative controls for the study.

2.3. Evaluation of skin conditions

To assess the skin condition of each mouse, we measured transepidermal water loss (TEWL) and stratum corneum (SC) hydration, which reflects the moisture content in the stratum corneum. We used Tewameter TM300 and Corneometer CM825 (Courage and Khazawa, Cologne, Germany) for these assessments. The measurements were conducted the day after generating the AEW-treated mice (day-7), prior to administration of reagents or antibodies. Some measurements were also taken the day before the first treatment in AEW-treated, W-treated, NT mice, respectively. Measurements were performed with the devices in contact with the rostral back, following anesthesia with sevoflurane. Each experiment included 8 mice per treatment group.

2.4. Preparation and administration of reagents and antibodies

We employed the following JAK inhibitors: baricitinib (MedChem Express, Monmouth Junction, NJ, USA), abrocitinib (Selleckchem, Houston, TX, USA), and AZ960 (Selleckchem) as a JAK1/2 inhibitor, JAK1 selective inhibitor, and JAK2 selective inhibitor, respectively. They were used at concentrations of 3 mg/kg, 15 mg/kg, and 1.58 mg/kg, respectively, based on equivalence of effective concentrations

determined by IC50 values (Table S1). Each JAK inhibitor was dissolved in dimethyl sulfoxide (DMSO, SIGMA, St. Louis, MO, USA) to a volume of 75 μL, then adjusted to a volume of 3 mL with methyl cellulose (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). A 2.5% DMSO in methyl cellulose solution (75 μL DMSO/2.925 mL methyl cellulose) was used as the negative control. All JAK inhibitors and their negative controls were orally administered at 100 μL per 10 g of body weight per mouse on the day after generating AEW-treated mice. For the preparation of recombinant cytokines, murine IL-4 and IL-13 (both from PeproTech, Cranbury, NJ, USA) and TSLP (R&D Systems, Minneapolis, MN, USA) were dissolved in phosphate-buffered saline (PBS) at concentrations of 200 pg/mL, 2 ng/mL, and 2 ng/mL, respectively. The concentrations of these cytokines were determined with previous report [9]. These recombinant cytokines and PBS (as the negative control) were administered intradermally (50 μL) to the rostral back of naïve mice. Neutralizing antibodies including rat anti-mouse IL-4, rat anti-mouse IL-13, and rat anti-mouse TSLP were all from Thermo-Fisher, Waltham, MA, USA. Isotype control antibodies (rat IgG1 kappa isotype control for IL-4 and IL-13 and rat IgG2a kappa isotype control for TSLP) were from Thermo-Fisher and used as appropriate negative controls. These antibodies were dissolved in PBS and administered to the rostral back of AEW-treated mice on day-7 (50 μL), at concentrations 500-fold higher than the

corresponding cytokines (i.e., 0.1 μg/mL for IL-4 neutralizing antibodies and 1 μg/mL for IL-13 and TSLP neutralizing antibodies) by intradermal injection. In all conditions, the m-alloknesis assay was initiated 30 min after the last dose was administered.

2.5. M-alloknesis assay

M-alloknesis assays were performed based on previously reported methods with slight modifications [6, 10]. Mice were shaved approximately 7-days (range, 6-8 days) prior to mechanical stimulation. On the day of the assay, each mouse was transferred to a new cage to acclimatize for at least 1 h. Mice that received JAK inhibitors, recombinant cytokines, or neutralizing antibodies, were given additional time as required for administration. After the acclimatization period (or administration), the shaved rostral back was stimulated using von Frey filaments (Bioseb, Chaville, France) for a total of 30 stimulations comprising 3 stimulations at more than 5 s intervals 10 times. M-alloknesis scores were calculated as the total number of scratching responses. For this test, both 0.07 g and 0.16 g von Frey filaments were utilized, although only the data from the 0.16 g filaments are presented. Each experiment included 7 or 8 mice per treatment group.

2.6. Scratch bout counting assay

Scratching bout counting assays were conducted following completion of the m-alloknesis assay, with observers outside the experiment room to avoid confounding variables. Each mouse was placed into acrylic cages (19.5 x 24 x 35 cm) divided into four compartments and allowed to acclimatize for 1 h before measurements. The number of scratching bouts on the rostral back during a 3 h period was analyzed using the SCLABA® system (NOVERTEC, Kobe, Japan). A single scratching behavior was defined as the period from when the mouse began scratching the rostral back with its hind limb to when it finished [11]. Each experiment included 8 mice per treatment group.

2.7. ProcartalPlex multiplex immunoassay

The levels of IL-4, IL-13, IL-31 and TSLP in mouse skin were quantified using ProcartalPlex multiplex immunoassay, following the manufacturer's protocol (Supplemental materials).

2.8. Statistical analysis

Each dataset underwent analysis using a t-test for comparisons between two groups or one-way ANOVA for multiple comparisons, followed by the Tukey-Kramer test. Statistical analyses and graphical representations were conducted using GraphPad Prism

9 (GraphPad Software Inc., San Diego, CA, USA). Results with p-values of 0.05 or less were deemed statistically significant.

3. Results

3.1. Characterization of dry skin model mouse: skin barrier condition, spontaneous itch, and m-alloknesis

We established an AEW mouse model of dry skin (henceforth referred to as AEW-treated mice), by subjecting them to a twice-daily regimen of a 1:1 mixture of acetone and ether, followed by water, for six consecutive days (the upper panel of Fig. 1a). For comparison, we also included water (W)-treated mice following the same time schedule and non-treated (NT) mice as two negative control groups (the middle and the bottom panels of Figure 1a, respectively). On day-7, the rostral back skin of AEW-treated mice exhibited pronounced dryness and redness compared to the NT- and W-treated mice (Fig. 1b). To assess the effectiveness of the model, we measured transepidermal water loss (TEWL) and stratum corneum (SC) hydration before and after each treatment, serving as indicators of skin dryness. Only the AEW-treated mice, on the day following treatment, displayed increased TEWL (Fig. 1c) and decreased SC hydration (Fig. 1d) compared to all other conditions, confirming the validity of the dry-skin model mouse and control

groups. Using these groups, we examined the frequency of spontaneous itch and m-alloknesis. AEW-treated mice showed significant increase in spontaneous scratching behavior on day-7 (Fig. 1f) compared to NT- and W-treated mice. Assessment of m-alloknesis, measured through 30 stimulations using von Frey filaments (m-alloknesis assay, Figure 1e), revealed a significantly higher m-alloknesis score in AEW-treated than NT- and W-treated mice (Fig. 1g).

3.2. Effects of oral JAK inhibitors on m-alloknesis in dry skin model mouse

We proceeded to investigate the impact of oral JAK inhibitors on both m-alloknesis and spontaneous scratching behavior in AEW-treated mice. We administered baricitinib, abrocitinib, and AZ960, representing an oral JAK1/2 inhibitor, an oral JAK1 selective inhibitor, and an oral JAK2 selective inhibitor, respectively. We conducted an m-alloknesis assay 30-min after each JAK inhibitor administration and then recorded the number of spontaneous scratching behaviors in the mice (Fig. 2a). As a result, all three JAK inhibitors significantly reduced m-alloknesis score. The JAK1 selective inhibitor demonstrated a stronger inhibitory effect on m-alloknesis in AEW-treated mice than the JAK2 selective inhibitor, while no significant difference was observed between the effect of the JAK1 selective inhibitor and that of the JAK1/2 inhibitor (Fig. 2b). However,

concerning spontaneous scratching behavior in AEW-treated mice, none of the JAK inhibitors showed significant suppression (Fig. 2c). These findings suggest the inhibitors were ineffective against spontaneous itch, while the JAK1 selective inhibitor exhibited the most potent inhibition of m-alloknesis in AEW-treated mice.

3.3. M-alloknesis in AEW-treated mice was inhibited by Interleukin (IL)-4, IL-13 and thymic stromal lymphopoietin (TSLP) neutralizing antibody

The JAK/signal transducer and activator of transcription (STAT) pathway plays a crucial role in the signal transduction of various cytokines [12, 13]. With this understanding, our focus turned to IL-4, IL-13, and TSLP, which are upstream components of the JAK/STAT pathway and directly implicated in itch induction [14-16]. Our aim was to discern the action points of the JAK1 inhibitor concerning m-alloknesis in the dry skin model. To investigate whether IL-4, IL-13, or TSLP played a role in m-alloknesis, we conducted m-alloknesis assays in AEW-treated mice in the presence of neutralizing antibodies for these cytokines. AEW-treated mice received intradermal administration of each neutralizing antibody (or PBS as a vehicle control), and 30-min later they underwent m-alloknesis assays (Fig. 3a). The m-alloknesis score in AEW-

treated mice was significantly suppressed by each neutralizing antibody targeting IL-4, IL-13, or TSLP compared to the vehicle control (PBS) (Fig. 3b-d).

3.4. M-alloknesis induced by IL-4, IL-13 or TSLP was significantly reduced by JAK1 selective inhibitor

We next examined whether type 2 cytokines induce m-alloknesis on their own. Recombinant murine IL-4, IL-13, or TSLP was administered to naïve mice via intradermal injection, and the m-alloknesis assay was conducted 30-min later (Fig. 4a). The found that each of the three cytokines induced m-alloknesis compared to a vehicle control (PBS) (Fig. 4b-d). Furthermore, we investigated the effect of the oral JAK1 selective inhibitor, abrocitinib, on m-alloknesis induced by IL-4, IL-13, or TSLP. The m-alloknesis assay was performed 30-mins following intradermal administration of IL-4, IL-13, or TSLP, with pretreatment using the oral JAK1 selective inhibitor or a vehicle control (DMSO) (Fig. 4e). The m-alloknesis score induced by IL-4, IL-13, or TSLP in naïve mice was significantly reduced by prior administration of the JAK1 inhibitor compared to the DMSO control (Fig. 4f-h).

3.5. TSLP is significantly increased in skin of AEW-treated mice

Finally, we collected skin samples from mice treated with NT, W, and AEW and measured cytokine levels using a highly sensitive quantitative assay (Luminex®). IL-4, IL-13, and IL-31 showed a trend towards an increase, but no significant difference was observed (Fig. 5a-c). However, TSLP was significantly increased in the skin samples of AEW-treated mice (Fig. 5d).

4. Discussion

In our study, m-alloknesis induced by dry skin was effectively inhibited by oral administration of the JAK1 selective inhibitor abrocitinib (Fig. 2b), whereas spontaneous itch remained unaffected (Fig. 2c). Our data demonstrated that neutralizing antibodies against IL-4, IL-13, and TSLP effectively inhibited m-alloknesis in the dry skin model (Fig. 3b-d). Additionally, our highly sensitive quantitative assay revealed a significant increase in TSLP levels in the skin of AEW-treated mice (Fig. 5d). While IL-4, IL-13, and IL-31 exhibited an increasing trend in the context of dry skin, the differences were not statistically significant (Fig. 5a-c). Taken together, these findings suggest the involvement of type 2 cytokine-JAK1 signaling in the development of dry skin-induced m-alloknesis (Fig. 6). Supporting this notion, we observed that type 2 cytokines (IL-4, IL-13, and TSLP) induced m-alloknesis in naïve mice (Fig. 4b-d) and that this response was effectively suppressed by the oral JAK1 selective inhibitor abrocitinib (Figure 4f-h). Consistent with previous reports [3, 17, 18], our AEW-treated mice exhibited increased TEWL and decreased SC hydration compared to the two control groups, W-treated and NT mice (Fig. 1c, d), indicating the development of dry skin after 6-days of AEW treatment. In this model, we re-evaluated two types of itch: spontaneous itch (Fig. 1f) and m-alloknesis (Fig. 1g). Both types of itch were significantly increased compared to

negative controls (Fig. 1f, g), consistent with previous reports [6, 17, 18]. These data indicated that these AEW-treated mice were successfully established as dry skin model to evaluate the inhibitory effects of JAK inhibitors.

From a behavioral pharmacology perspective, we observed that among the JAK inhibitors evaluated in this study, abrocitinib, a JAK1-selective inhibitor, exhibited the most potent inhibition of m-alloknesis in AEW-treated mice (Fig. 2b). These findings suggest that both JAK1 and JAK2, with a stronger emphasis on JAK1, play pivotal roles in inducing m-alloknesis in the context of dry skin. Immunohistochemical staining revealed increased expression levels of both total protein and phosphorylated JAK1 (Supplementary Fig. 1a-f) and phosphorylated JAK2 (Supplementary Fig. 2a-d) in the epidermis of AEW-treated mice compared to NT mice. Furthermore, phosphorylated JAK1 (Supplementary Fig. 1h) and JAK2 (Supplementary Fig. 2e) were clearly detected in intraepidermal nerve fiber-like structures in AEW-treated mice, strongly suggesting JAK1 and JAK2 activation in epidermal nerves. These findings highlight the significance of JAKs in this model.

Returning to Fig. 2b, despite the theoretical similarity in JAK1 inhibitory activity between the JAK1/2 inhibitor (baricitinib) and the JAK1-selective inhibitor (abrocitinib) based on IC50 values, the inhibitory effect on m-alloknesis differed in that no significant

difference was observed compared to that of JAK2 selective inhibitor (AZ960) (Fig. 2b). This discrepancy may be due to the competitive binding of baricitinib to JAK2, which reduces its binding frequency to JAK1, lowering its effective JAK1 inhibitory activity and diminishing its effect on m-alloknesis.

Conversely, our results indicate that none of these JAK inhibitors effectively addressed the issue of spontaneous itch in AEW-treated mice (Fig. 2c). This could be partially explained by a previous study suggesting that IL-33 signaling, with MyD88 as a downstream adapter protein unaffected by JAK inhibitors, is involved in the amplification of spontaneous itch in dry skin [19]. Although it is impossible to be certain without inhibiting the function of IL-33, it is possible that in dry skin, the IL-33-MyD88 axis contributes more to the induction of spontaneous itch than to m-alloknesis.

Our present data strongly indicate that IL-4, IL-13, and TSLP play a significant role in inducing m-alloknesis (Fig. 3, 4). These findings agree with prior research [9]. Additionally, IL-4 and IL-13 have been reported to directly induce itch [16]. Even small amounts of IL-4 have been shown to sensitize neuronal subsets to various pruritogens, reducing the itch threshold, a phenomenon known as neuronal sensitization [14]. Furthermore, a previous study reported that intradermal injection of TSLP directly induced itch by activating its cognate receptor on sensory nerve fibers in mice [15]. Also,

a TSLPR inhibitor was demonstrated to suppress scratching behavior in a dry skin model [20]. Given the small dose administered in this study, it is possible that IL-4, along with IL-13 and TSLP, which can directly affect nerves via the JAK/STAT pathway, may induce m-alloknesis in dry skin by sensitizing nerves (Fig. 6). Using Nav1.8-expressing neuron-specific JAK1-deficient mice, it has been reported that IL-4-induced neuronal sensitization is mediated by JAK1-positive neurons [14]. Since our immunohistochemical results showed JAK1 and JAK2 phosphorylation in nerve fiber-like structures in AEW-treated mice (Supplemental Fig. 1h and 2e), it is strongly speculated that alloknesis induction in this model is also mediated through JAK1- or JAK2-positive nerves.

We could not identify the specific peripheral nerve subpopulation responsible for m-alloknesis. Although Nav1.8 is preferentially expressed in C-fibers, approximately 40% of Nav1.8-positive neurons are also Aδ- or Aβ-fibers [21]. Since PIEZO1-positive C-fibers [22] and TLR5-positive Aβ-fibers [23] have been implicated in mediating m-alloknesis, it is possible that a subpopulation of these neuronal types expressing JAK1 (and JAK2) is sensitized by IL-4, IL-13, and TSLP, leading to an altered perception of weak mechanical stimuli as itch, thereby inducing m-alloknesis (Fig. 6).

Our highly sensitive quantitative assay confirmed a significant increase in TSLP levels in AEW-treated mice (Fig. 5d). Although no significant difference was observed

and levels were close to the detection limit, similar to a previous report [9], IL-4, IL-13, and IL-31 displayed an upward trend in the context of dry skin (Fig. 5a-c). This increase in TSLP levels agrees with findings of another study [20]. TSLP is known to induce IL-4, IL-13, and IL-31 [24-26]. While it cannot be definitively determined due to each cytokine's optimal concentration, these results suggest TSLP plays an important role in the induction of m-alloknesis. However, the observation that mice with elevated levels of IL-4, IL-13, and IL-31 also had increased levels of TSLP, and that inhibitory antibodies effectively reduced m-alloknesis (Fig. 3b-d), supports the previous claim that even small amounts of IL-4 and IL-13 are involved in the induction of m-alloknesis in this model [9]. As a limitation, this study was unable to obtain further information about IL-31 since a neutralizing antibody against murine IL-31 was unavailable. Nevertheless, it was reported that IL-31 could induce m-alloknesis [27], implying that IL-31, as well as the other cytokines in this study, may contribute to the induction of mechanical alloknesis in dry skin. These findings raise the possibility that IL-31, along with the other cytokines studied, contributes to the induction of m-alloknesis in dry skin.

Previous reports have suggested that transcription levels of *tslp* are upregulated in keratinocytes of AEW-treated skin [20] This finding, along with research indicating that TSLP from keratinocytes plays a crucial role in the early stages of atopic dermatitis [25,

26], suggests that keratinocytes are likely the primary source of TSLP. Additionally, previous results showed increased eosinophils and basophils in AEW-treated mice [9], suggesting that these granulocytes might be sources of IL-4 and IL-13. However, it has also been reported that TSLP can promote the production of IL-4 and IL-13 by activating Th2 cells [26] or group-2 innate lymphoid cells (ILC2) [28]. Consequently, while this study did not aim to identify the specific sources of these cytokines, it's plausible that dermal ILC2 and a small population of Th2 cells induced in the early stages of inflammation, in line with the concept of type-2 inflammation expansion by ILC2 resident in the skin [29], may contribute to the production of IL-4 and IL-13 (Fig. 6).

In conclusion, the findings of this study reveal that all the oral JAK1/2 inhibitors, JAK1 selective inhibitors, and JAK2 selective inhibitors demonstrate the ability to alleviate m-alloknesis in AEW-treated dry skin model mouse, with JAK1 selective inhibitors showing the most pronounced effect. Our results suggest that JAK1 inhibitor suppresses m-alloknesis by inhibiting neuronal sensitization of TSLP, IL-4, and IL-13 through blockade of their downstream signal transduction via the JAK/STAT pathways. While further investigation is necessary, our findings suggest TSLP may play a central role among these cytokines. These insights hold promise for the management of m-alloknesis in dry skin-associated conditions, including xerosis and AD.

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Conflicts

The authors have no conflicts of interest to declare.

CRediT authorship contribution statement

Conceptualization: K.T.; Funding acquisition: K.T., E.K. and M.T.; investigation: Y.T. and E.K.; methodology: E.K. and M.T.; project administration: E.K. and M.T.; supervision: M.T. and K.T.; writing-original draft preparation: Y.T, E.K. and M.T.; writing-review and editing: T.K., Y.S., and K.T.; All authors have read and agreed to the published version of the manuscript.

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Appendix A. Supporting information

Supplemental materials associated with this article can be found in the online version.

Figure legends

Fig. 1. Establishment and characterization of acetone-ether and water (AEW)-treated dry skin model mouse. (a) Schema of procedure to establish the dry skin model and controls. (b) Representative images of shaved mice. (c) Transepidermal water loss (TEWL) and (d) stratum corneum (SC) hydration recorded in the rostral back of AEW-treated, water (W)-treated, and non-treated (NT) mice on day 7. n=8 mice for each. (e) Schema of procedure conducted on day 7 to assess m (mechanical)-alloknesis and spontaneous itch. (f and g) Frequency of scratching bouts in mice during 3 h (spontaneous itch) (f) and m-alloknesis scores (g) of NT, W-treatment, and AEW-treatment mice on day 7. n=8 for each. Mean ± standard error of the mean for each group. Bf: Before treatment, Af: after treatment, *: $p < 0.05$, **: $p < 0.01$.

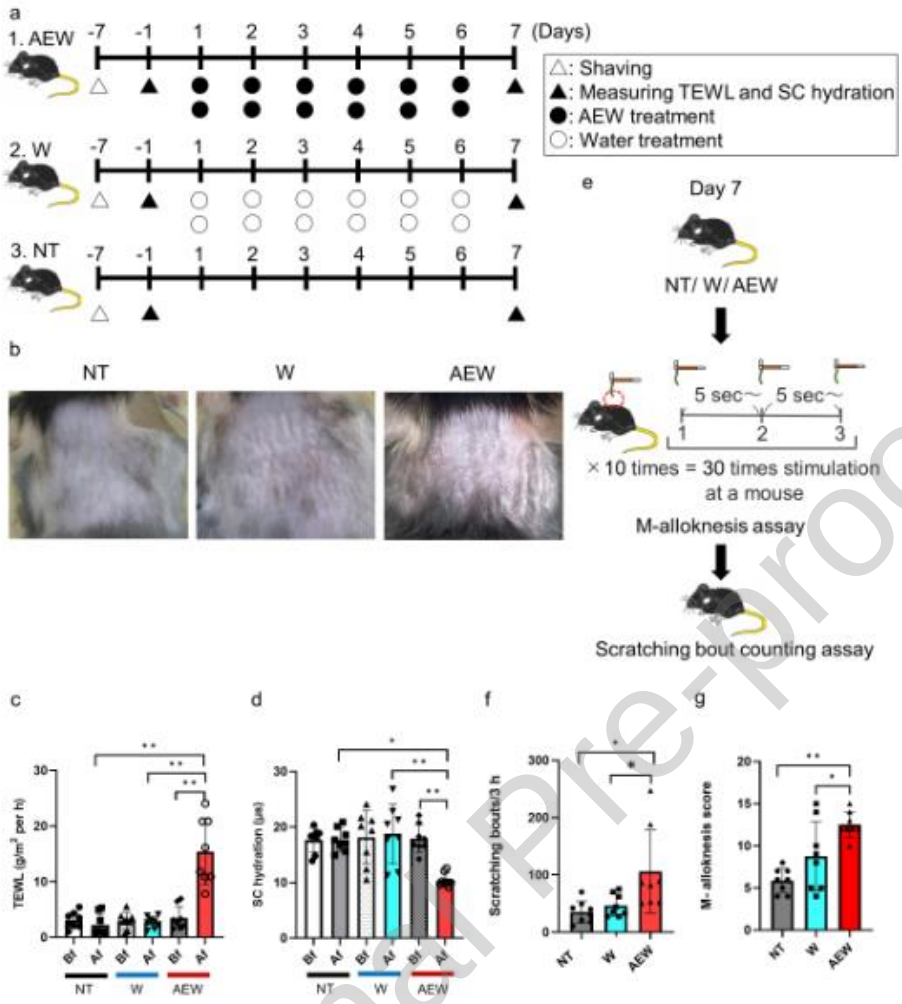


Fig. 2. Effects of oral Janus kinase (JAK) inhibitors on m-alloknesis and spontaneous scratching behavior in dry skin model mice. (a) Schematic procedure of m-alloknesis assay and scratching bout counting assay following various oral JAK inhibitors: JAK1/2 (baricitinib), JAK1 selective inhibitor (abrocitinib), and JAK2 selective inhibitor (AZ960) in AEW-treatment mice. (b) Effects of various oral JAK

inhibitors on m-alloknesis. n=8 mice for each. (c) Effects on the frequency of scratching bouts during 3 h with no mechanical stimulation. n=8 for each. Mean ± standard error of the mean for each group. p.o.: per os, *: $p < 0.05$, **: $p < 0.01$.

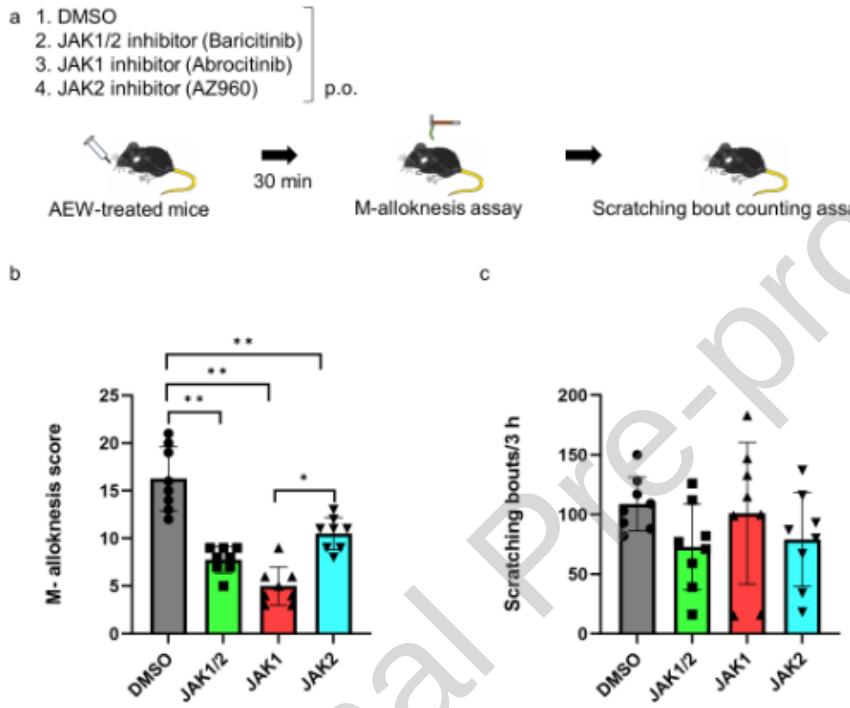


Fig. 3. Effects of neutralizing antibodies against interleukin (IL)-4, IL-13 or thymic stromal lymphopoietin (TSLP) on m-alloknesis in dry skin model mice. (a) Schematic depicting the m-alloknesis assay in AEW-treatment mice following the administration of neutralizing antibodies against type 2 T helper (Th2) cytokines: IL-4, IL-13, or TSLP. (b)-(d) Effects of neutralizing antibodies (Abs) against IL-4 (b), IL-13 (c), or TSLP (d) on m-

alloknesis in AEW-treatment mice. The IL-13Ab treatment group comprised seven mice, and the other groups eight mice each. Mean ± standard error of the mean for each group. i.d.: intradermal, **: $p < 0.01$.

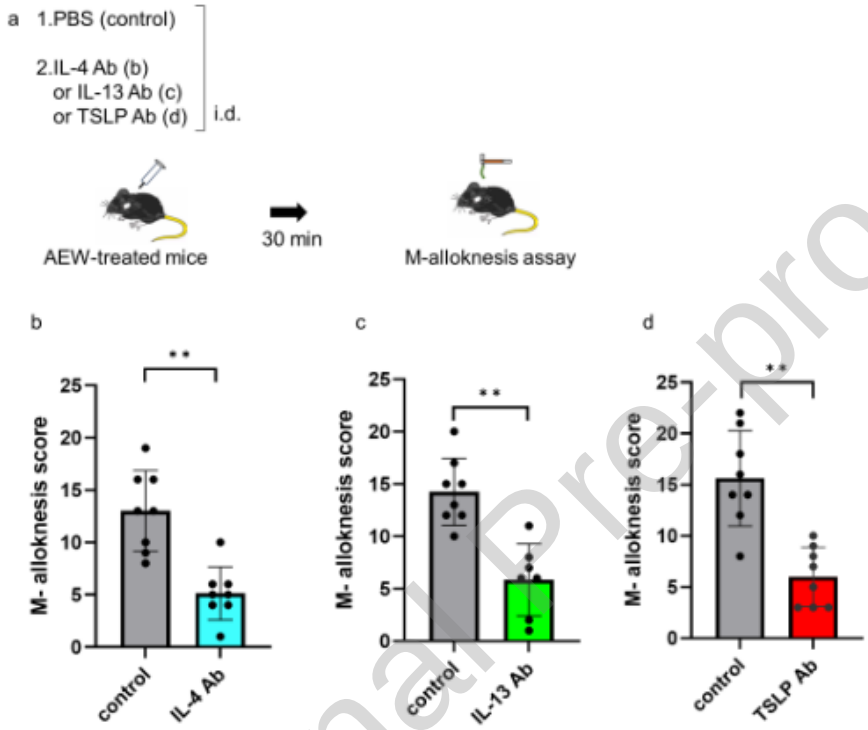


Fig. 4. Effects of JAK1 inhibitor on IL-4, IL-13 and TSLP-induced m-alloknesis. (a) Schematic procedure for the m-alloknesis assay following intradermal administration of various pruritogenic Th2 cytokines. (b)-(d) Effects of various pruritogenic Th2 cytokines: IL-4 (b), IL-13 (c) or TSLP (d) on m-alloknesis in naïve mice. (e) Schematic procedure of m-alloknesis assay following above Th2 cytokines, under the pretreatment of JAK1

selective inhibitor (abrocitinib). (f)-(h) Effects of abrocitinib on m-alloknesis induced by IL-4 (f), IL-13 (g) or TSLP (h) via intradermal injection in naïve mice. n=8 mice for each. Mean ± standard error of the mean for each group. i.d.: intradermal, p.o.: per os, *: $p < 0.05$, **: $p < 0.01$.

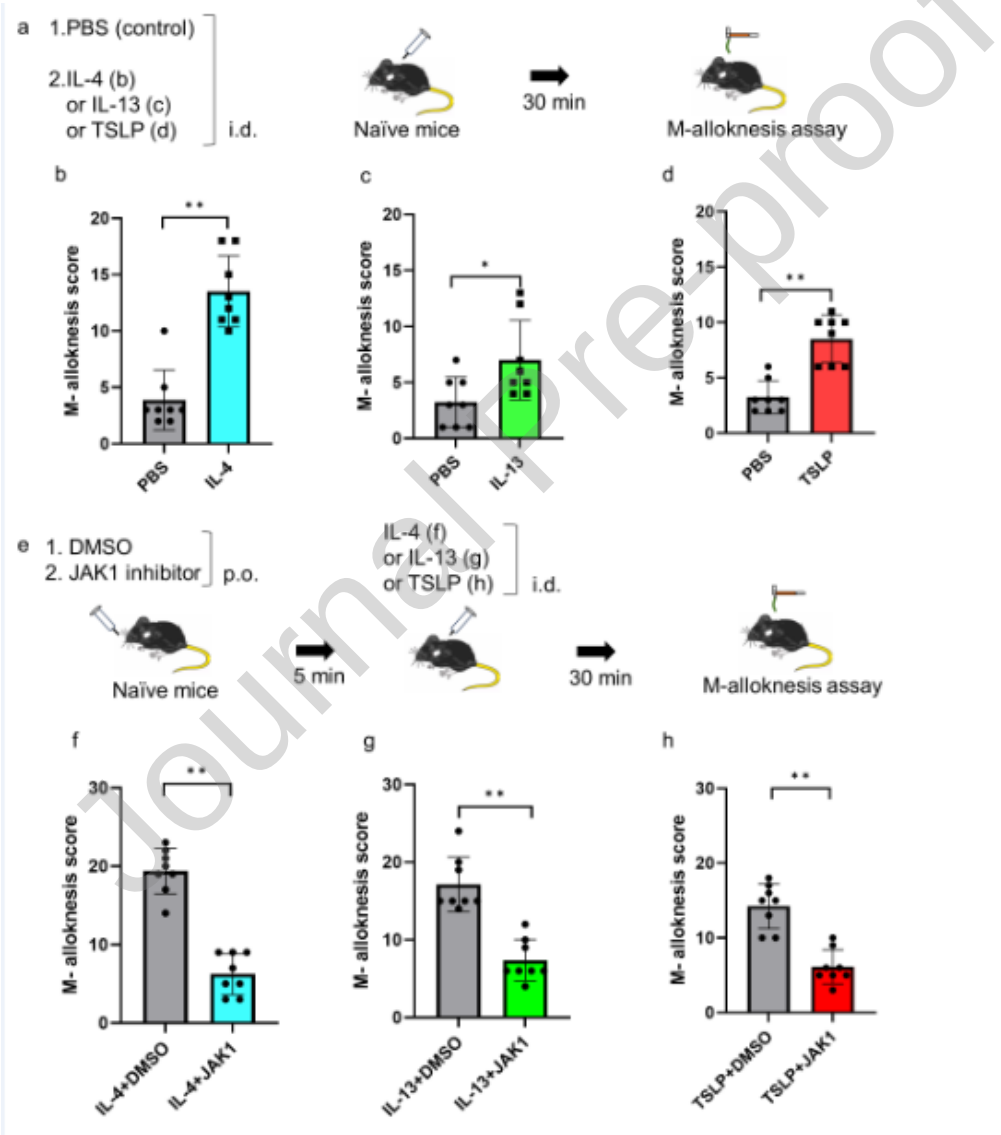


Fig. 5. Measurement of IL-4, IL-13, IL-31 and TSLP levels in dry skin model mice. (a)-(d) The levels of IL-4 (a), IL-13 (b), IL-31 (c), and TSLP (d) in skin homogenate of NT, W-treatment, and AEW-treatment mice were determined by highly sensitive cytokine quantification method (ProcartalPlex Multiplex Immunoassay). n=6 mice for each. Mean±standard error of the mean of each group. N.D.: not detected, ns: no significant, **: $p<0.01$.

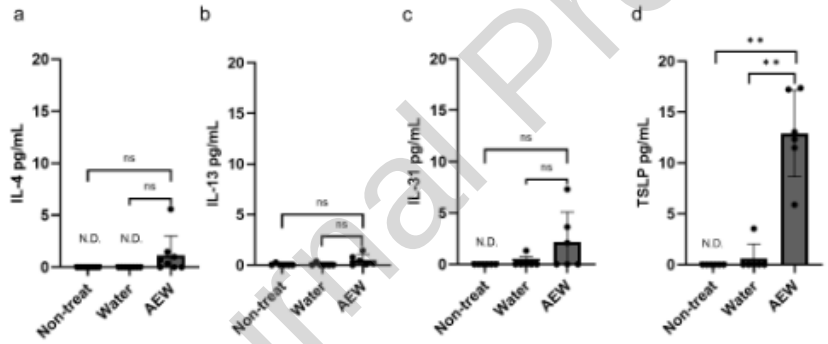
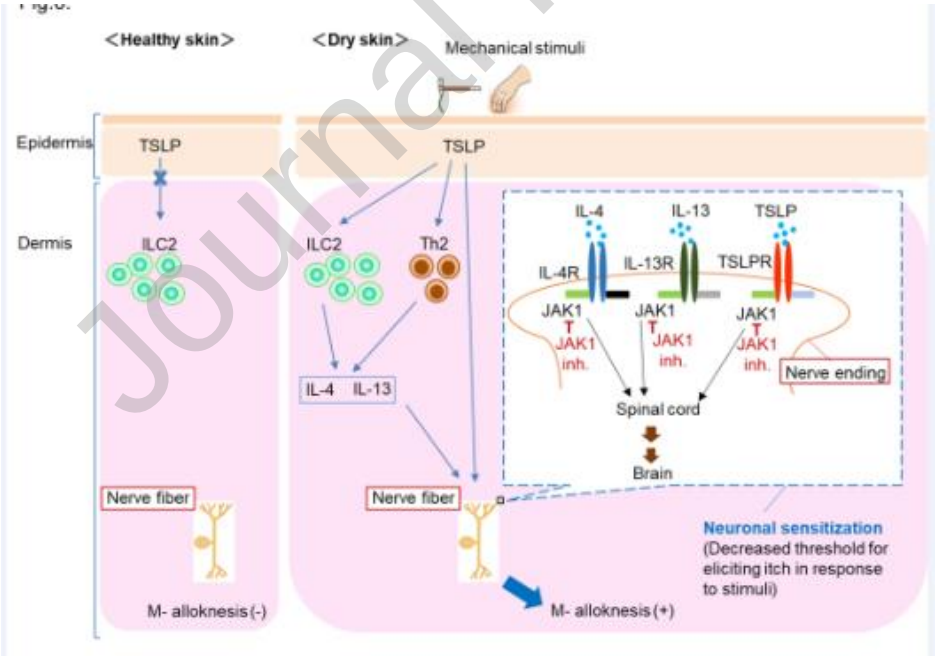


Fig. 6. Schematic diagram of induction mechanism of m-alloknesis in dry skin. Left panel: In healthy skin, as the skin barrier is well-maintained, TSLP is exist within the keratinocytes. Therefore, skin-resident ILC2 does not activate Th2 inflammation and m-alloknesis does not occur. Right panel: In dry skin, as the skin barrier is disrupted,

TSLP may be easily released from keratinocyte to dermis by mechanical stimuli such as scratching behavior. Released TSLP may promote the production of IL-4 and IL-13 directly through ILC2 activation, leading to the initiation of Th2 inflammation. TSLP can also activates Th2 cells to produce these cytokines by ILC2 independent pathway. TSLP, IL-4, and IL-13 may induce m-alloknesis through neuronal sensitization, making it easier to evoke itch by mechanical stimulation from such as von Frey filaments. As JAK1 is thought to be located downstream of the signaling pathways of Th2 cytokines, the JAK1 inhibitor in this study may suppress m-alloknesis by inhibiting Th2 cytokines-JAK1 signaling neuronal sensitization pathway at nerve terminals.



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CRediT authorship contribution statement

Conceptualization: K.T.; Funding acquisition: K.T., E.K. and M.T.; investigation: Y.T. and E.K.; methodology: E.K. and M.T.; project administration: E.K. and M.T.; supervision: M.T. and K.T.; writing-original draft preparation: Y.T, E.K. and M.T.; writing-review and editing: T.K., Y.S., and K.T.; All authors have read and agreed to the published version of the manuscript.

Highlights

- Mechanical (m)-alloknesis is itch hypersensitivity by harmless mechanical stimuli.
- M-alloknesis often triggers itch-scratch cycle in dry-skin based skin diseases.
- Both JAK1 and 2, especially JAK1 inhibitor inhibited m-alloknesis in dry skin model.
- IL-4, IL-13, and TSLP induced m-alloknesis, which were inhibited by JAK1 inhibitor.

- In the model skin, Th2 cytokines were detected with a significant increase in TSLP.
- Th2 cytokines-JAK1 signaling may induce m-alloknesis by neuronal sensitization.