

Accepted Manuscript

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PII: S0003-9861(13)00363-9

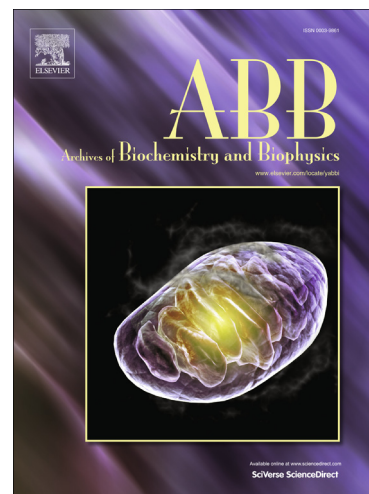
DOI: <http://dx.doi.org/10.1016/j.abb.2013.11.010>

Reference: YABBI 6571

To appear in: *Archives of Biochemistry and Biophysics*

Received Date: 18 September 2013

Revised Date: 5 November 2013



Please cite this article as: N-R. Han, P-D. Moon, H-M. Kim, H-J. Jeong, Tryptanthrin ameliorates atopic dermatitis through down-regulation of TSLP, *Archives of Biochemistry and Biophysics* (2013), doi: <http://dx.doi.org/10.1016/j.abb.2013.11.010>

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Tryptanthrin ameliorates atopic dermatitis through down-regulation of TSLP**Na-Ra Han^a, Phil-Dong Moon^a, Hyung-Min Kim^{*,a}, Hyun-Ja Jeong^{*,b}**

^a*Department of Pharmacology, College of Korean Medicine, Kyung Hee University, 1 Hoegi-dong, Dongdaemun-gu, Seoul, 130-701, Republic of Korea*

^b*Biochip Research Center and Inflammatory Disease Research Center, Hoseo University, 165, Sechul-ri, Baebang-myun, Asan, Chungnam 336-795, Republic of Korea*

Corresponding author. Address: Biochip Research Center and Inflammatory Disease Research Center, Hoseo University, 165, Sechul-ri, Baebang-myun, Asan, Chungnam 336-795, Korea, Tel.: +82-41-540-9681, Fax: +82-41-542-9681, *E-mail address*: hjjeong@hoseo.edu (H.J. Jeong) or College of Oriental Medicine, Kyung Hee University, 1 Hoegi-dong, Dongdaemun-gu, Seoul 130-701, South Korea, Tel.: +82-2-961-9448, Fax: +82-2-967-7707, *E-mail address*: hmkim@khu.ac.kr (H.M. Kim).

Abbreviations used: AD, atopic dermatitis; DNFB, 2,4-dinitrofluorobenzene; HMC-1, human mast cell line; PMACI, phorbol myristate acetate/calcium ionophore A23187; TR, tryptanthrin; TSLP, Thymic stromal lymphopoietin.

ABSTRACT

Atopic dermatitis (AD) is a common skin disease that greatly worsens quality of life. Thymic stromal lymphopoietin (TSLP) plays a decisive role in the development of AD. The purpose of this study is to examine whether tryptanthrin (TR) would suppress AD through the regulation of TSLP. We analyzed the effect of TR on the level of TSLP from phorbol myristate acetate/calcium ionophore A23187-activated human mast cell line, HMC-1 cells, in 2,4-dinitrofluorobenzene-induced AD-like skin lesions of NC/Nga mice, and in anti-CD3/anti-CD28-stimulated splenocytes. TR significantly suppressed the level of intracellular calcium and the production and mRNA expression of TSLP through the blockade of receptor-interacting protein 2/caspase-1/nuclear factor- κ B pathway in the activated HMC-1 cells. TR also significantly suppressed the levels of histidine decarboxylase and IL-1 β . Furthermore, TR ameliorated clinical symptoms in the AD model. TR significantly reduced the levels of TSLP, IL-4, IFN- γ , IL-6, TNF- α , thymus and activation-regulated chemokine, and caspase-1 in AD skin lesions. Also, TR significantly reduced the serum levels of histamine and IL-4 in the AD model. Finally, TR significantly inhibited the production of IL-4, IFN- γ , and TNF- α from the stimulated splenocytes. Taken together, TR exhibits the potential to be a therapeutic agent for AD through down-regulation of TSLP.

Keywords: Tryptanthrin , TSLP, Caspase-1, Mast cell, Atopic dermatitis

Introduction

Atopic dermatitis (AD) is one of the most common inflammatory cutaneous diseases, characterized by dry, itchy skin, and relapsing eczematous skin lesions [1]. The etiology of this disease is incompletely understood, but the disease is manifested by complex interactions between various cells and molecular mediators. Mast cells as effector cells of allergic reactions are involved in the development of skin lesions in AD [2]. Mast cells secrete numerous cytokines that are relevant in chronic skin inflammation [3]. Thymic stromal lymphopoietin (TSLP), an interleukin 7-like cytokine, has been mainly known to trigger dendritic cell-mediated Th2 inflammatory responses [4]. However, the activated mast cells have expressed high levels of TSLP and triggered allergic inflammation [5]. And TSLP was highly expressed in skin lesions of patients with acute and chronic AD [6].

The activated inflammasome by an allergen causes the activation of caspase-1 in a cytosolic multi-adaptors complex, inflammasome-specific manner, which in turn drives maturation and secretion of the pro-inflammatory cytokines and regulates innate and adaptive immune responses [7]. Caspase-1 is responsible for activating the inactive precursor of interleukin (IL)-1 β that are critical for inflammation [8]. Rereceptor-interacting protein 2 (Rip2) regulates caspase-1 which is activated within the inflammasome [9]. And Rip2 activates the transcription factor, nuclear factor (NF)- κ B pathway that mediates immune and inflammation responses [10]. Caspase-1 is able to activate NF- κ B via Rip2 [11]. NF- κ B regulates the transcription of the TSLP gene by binding to its promoter [12].

The skin lesions in NC/Nga mice of murine AD models are characterized by

infiltration of IL-4-producing CD4⁺ T cells or mast cells as well as high expressions of Th2 cytokines and chemokines [13]. And repeated skin exposure to the haptens, such as 2,4-dinitrofluorobenzene (DNFB) causes AD-like skin lesions [14]. DNFB was reported to increase the caspase-1 activity in mouse skin dendritic cells [15].

Tryptanthrin (TR, Indolo(2,1-b)quinazoline-6,12-dione) is a natural product from the medicinal plant, *Polygonum tinctorium* (Naju Jjok) which is known to have anti-pyretic, anti-inflammatory, and detoxicant actions in traditional Korea medicine [16,17]. TR has been revealed to have therapeutic effects on inflammatory bowel disease [17], tuberculosis [18], and *Escherichia coli* infection [19]. However, the effect of TR on AD has not yet been clarified. Therefore, we investigated the effect of TR on phorbol myristate acetate/calcium ionophore A23187 (PMACI)-stimulated human mast cell line (HMC-1) cells in vitro, DNFB-induced AD-like skin lesions of NC/Nga mice in vivo, and anti-CD3/anti-CD28-stimulated splenocytes ex vivo.

Materials and methods

HMC-1 cells culture

HMC-1 cells were incubated in IMDM supplemented with 100 units/ml of penicillin, 100 µg/ml of streptomycin, and 10% fetal bovine serum (FBS) at 37 °C in 5% CO₂ with 95% humidity.

Animals

Male NC/Nga mice were obtained from Charles River Laboratories International, Inc. (Yokohama, Japan). And the animals were maintained under conventional condition and performed under approval from the animal care committee of Kyung Hee University [Protocol Number. KHUASP (SE)-11-009]. Mice were sacrificed with CO₂ inhalation.

Sensitization with DNFB

For active sensitization, 100 µl 0.15% DNFB dissolved in acetone was topically challenged to the shaved abdominal skins of NC/Nga mice. A week later, the shaved dorsal skins of NC/Nga mice was challenged with 50 µl 0.15% DNFB. At that time, TR (10 µM), DEX (3 µg/ml), or saline (control group) were orally administrated to DNFB-challenged mice (Supplementary Fig. S1). The same volume of acetone was challenged

to the shaved dorsal skin and saline was orally administrated as an unchallenged vehicle group. Dorsal skin samples, serum, and spleen were obtained 4 h after the last DNFB challenge. After anesthetization, blood was withdrawn from the heart of mouse into syringes. And then, serum was prepared by centrifugation at 3400 rpm at 4 °C for 10 min.

Statistical analysis

The results shown are a summary of the data from at least-three experiments and are presented as the mean $\pm$ SD. Statistical evaluation of the results was performed by an independent *t*-test and ANOVA with Tukey post hoc test. The results were considered significant at a value of $P < 0.05$.

Results

TR suppresses the level of TSLP from the activated HMC-1 cells

An increase in concentration of intracellular calcium is a sufficient condition for activation of mast cells and secretion of mediators from the mast cells [20]. Thus, first we examined the regulatory effect of TR on the level of intracellular calcium in the PMACI-activated HMC-1 cells. BAPTA-AM (calcium chelator) was used as a positive control. The activation with PMACI increased the calcium release from intracellular stores (in 0.5 mM EGTA containing media), whereas TR suppressed the level of intracellular calcium increased by PMACI (Fig. 1a). Because increased intracellular calcium induces the production of TSLP from the activated mast cells [21], we examined the regulatory effect of TR on the production of TSLP. TR (1 and 10 μ M) significantly suppressed the production of TSLP from the activated HMC-1 cells ($P < 0.05$; Fig. 1b). TR (1 and 10 μ M) also significantly reduced the mRNA expression of TSLP in the activated HMC-1 cells ($P < 0.05$; Fig. 1c). TR alone had no effect on the mRNA expression and production of TSLP (Fig. 1b and c). TR (0.1, 1, and 10 μ M) did not show cytotoxicity in the HMC-1 cells (Fig. 1d).

TR suppresses the activations of Rip2/caspase-1/NF- κ B in the activated HMC-1 cells

Next, we examined that the regulatory effect of TR on the level of TSLP that would be mediated through Rip2/caspase-1/NF- κ B pathways. As shown in Fig. 2a and b, TR significantly suppressed the expressions of Rip2 and caspase-1 that were increased

by activation with PMACI ($P < 0.05$). And TR significantly suppressed the caspase-1 enzymatic activity in a dose-dependent manner ($P < 0.05$; Fig. 2c). In addition, TR significantly suppressed the cleavage of the caspase-specific peptide (tetrapeptide WEHD-pNA, substrate of caspase-1) through inhibition of binding reaction between the recombinant caspase-1 and substrate during the indicated time ($P < 0.05$; Fig. 2d). Furthermore, TR suppressed the NF- κ B translocation to the nuclei and I κ B α phosphorylation in cytosol ($P < 0.05$; Fig. 2e and f). Caspase-1 was known as IL-1 β -converting enzyme and NF- κ B was a transcription factor of IL-1 β [8]. Therefore, we analyzed whether TR also would regulate the level of IL-1 β . TR significantly suppressed the production and mRNA expression of IL-1 β in the activated HMC-1 cells ($P < 0.05$; Fig. 3).

TR suppresses the level of HDC in the activated HMC-1 cells

Mast cells induce histamine release by increasing intracellular calcium [22]. Histamine was formed from L-histidine by activation of HDC [23]. Thus, we examined the effect of TR on HDC activity. Activation with PMACI increased the expression of HDC, whereas TR decreased the expression of HDC ($P < 0.05$; Supplementary Fig. S2a and b). And TR significantly suppressed the activity of HDC in the activated HMC-1 cells ($P < 0.05$; Supplementary Fig. S2c).

TR ameliorates clinical symptoms in DNFB-induced AD murine model

Furthermore, we examined the regulatory effect of TR on the TSLP level of the DNFB-induced AD murine model. DEX was used as a positive control [24]. The noticeable erythema, hemorrhage, excoriation, dryness, and erosion were present in DNFB-induced AD-like skin lesions (control group), whereas the administration of TR (10 μ M) or DEX (3 μ g/ml) markedly ameliorated these phenotypes in AD skin lesions (Fig. 4a). And we examined the the thickness of epidermis and infiltration of inflammatory cells by H&E staining. The epidermis thickness was reduced in skin lesions of TR or DEX-administered AD murine model (Fig. 4a). As shown in Fig. 4a and Supplementary Fig. S3, the number of inflammatory cells was lower in skin lesions of TR or DEX-administered AD murine model than that of the skin lesions of the control group ($P < 0.05$). In addition, TR or DEX significantly suppressed scratching behavior (Fig. 4b).

TR suppresses the level of TSLP in skin lesions of DNFB-induced AD murine model

Next, we examined whether TR would suppress the levels of TSLP in skin lesions of the AD murine model. The mRNA expression of TSLP was up-regulated by DNFB challenge, but the up-regulated mRNA expression was suppressed by TR (10 μ M) or DEX (3 μ g/ml) (Fig. 5a). The protein expression of TSLP was also significantly suppressed by TR or DEX ($P < 0.05$; Fig. 5b). In in vitro study, TR significantly suppressed the level of TSLP through the inhibition of caspase-1 activation in the HMC-1 cells. Thus, we examined whether TR would suppress the level of caspase-1 in vivo. DNFB challenge increased the activity of caspase-1, whereas TR (10 μ M) or DEX (3 μ g/ml) significantly suppressed the activity of caspase-1 in AD skin lesions ($P < 0.05$;

Fig. 5c). Histological analyses revealed that TR or DEX suppressed the mast cell-derived caspase-1 expression in AD skin lesions (Fig. 5d).

TR suppresses the levels of AD-mediated factors in DNFB-induced AD murine model

Furthermore, the mRNA expressions of IL-4, IFN- γ , IL-6, TNF- α , and TARC were suppressed by TR (10 μ M) or DEX (3 μ g/ml) (Fig. 6a and b). The protein expressions of IL-4 and IL-6 were also significantly suppressed by TR or DEX ($P < 0.05$; Fig. 6c). And TR or DEX significantly suppressed the levels of serum histamine and IL-4 ($P < 0.05$; Fig. 6d and e). However, TR or DEX did not significantly suppress the level of serum IgE (Fig. 6d).

TR suppresses the productions of cytokines from the stimulated splenocytes

Finally, we examined whether TR would suppress the production of IL-4, IFN- γ , TSLP, and TNF- α from the anti-CD3/anti-CD28 antibodies-stimulated splenocytes. As shown in Supplementary Fig. S4, anti-CD3/anti-CD28 antibodies increased the production of IL-4, IFN- γ , and TNF- α , whereas TR significantly suppressed the production of IL-4, IFN- γ , and TNF- α from the anti-CD3/anti-CD28 antibodies-stimulated splenocytes ($P < 0.05$; Supplementary Fig. S4). Stimulation with anti-CD3/anti-CD28 antibodies also induces the production of TSLP from splenocytes. However, TR did not significantly suppress the production of TSLP from the stimulated splenocytes (Supplementary Fig. S4).

Discussion

AD is one of the most common, chronic, and severe inflammatory skin diseases. Although topical steroids, emollients, and oral anti-histamines are used as the first-line therapy for AD, the long term use of topical steroids has induced adverse effects in many patients [25]. Thus, it is necessary to develop new drugs with novel actions that do not have the noticeable adverse effects. Natural product-based anti-inflammatory drugs with good efficacy and lower risk of adverse effects offer promising treatment and prevention of aggravating AD for patients [26]. In addition, herbal derivatives have been revealed to have therapeutic effects on AD in various experimental and clinical studies [27,28]. *Polygonum tinctorium* (Naju Jjok) has been used to treat inflammation, allergy, and microbial infection in traditional Korea medicine [29,16,30]. In this study, we showed that TR, a constituent of *Polygonum tinctorium*, also has therapeutic effects on AD in in vitro, in vivo, and ex vivo models.

Mast cells are inflammatory cells that respond to signals of innate and adaptive immunity [31]. The mast cells are increased in a majority of AD patients and involved in the pathogenesis of AD [32]. An increase in the intracellular calcium level has been proposed as an essential trigger for the activation of mast cells [33]. The activated mast cells release inflammatory mediators such as TSLP [34]. The level of TSLP was reduced through the blockade of intracellular calcium pathway in mast cells [21]. TSLP was significantly diminished in mast cell-deficient mice [35]. The expression of TSLP was highly increased in AD patients compared to healthy subjects [36]. Thus, we examined whether TR would regulate the level of TSLP through the modulation of calcium in mast cells and the AD murine model. TR suppressed the level of intracellular

calcium in the activated HMC-1 cells. TR suppressed the production and mRNA expression of TSLP on the activated HMC-1 cells. Also, TR suppressed the mRNA and protein level of TSLP in DNFB-induced AD-like skin lesions. DEX, which has been used as a positive control in vivo of this study, had been reported to have an inhibitory effect on TSLP in mast cells [37]. Thus, we can infer that TR would be beneficial for treating AD aggravated by increased TSLP.

Activation of inflammasome is associated with chronic inflammatory diseases [38]. Multiple key proteins of inflammasomes contain caspase recruitment domains (CARDs) [39]. The inflammasome is a multiprotein complex that mediates the activation of caspase-1 [40]. Quite unlike the role that most caspases have in apoptosis, caspase-1 mainly serves to cleave IL-1 β and IL-18 from their inactive precursors to their active forms [41]. However, it was revealed that caspase-1 activates NF- κ B and is involved in the production of the various inflammatory cytokines [42]. TSLP was expressed and produced through caspase-1 and NF- κ B in mast cells [43]. Caspase-1 transgenic mice overexpressing caspase-1 spontaneously suffered from chronic dermatitis and had a significant elevation in the serum levels of histamine and IgE [44,45]. Contact hypersensitivity to DNFB was inhibited in caspase-1-deficient mice [46]. Rip2 is an adapter protein of several adaptors comprising the inflammasome [9]. Rip2 CARD specifically interacts with other CARD-containing proteins such as caspase-1 [47]. Overexpression of Rip2 also leads to activation of the transcription factor NF- κ B [48]. Macrophages from Rip2-deficient mice showed impaired NF- κ B activation and reduced cytokine production in response to lipopolysaccharide stimulation [49]. In this study, TR suppressed the expressions of caspase-1 and Rip2 as well as caspase-1 enzymatic activity in the activated HMC-1 cells. TR suppressed the

levels of NF- κ B and pI κ B α . TR also suppressed the levels of IL-1 β in the activated HMC-1 cells. In addition, TR suppressed the activity and expression of caspase-1 in DNFB-induced AD-like skin lesions. Thus, these strong evidences support the proposal that TR has an anti-AD effect by regulating TSLP through the blockade of Rip2/caspase-1/NF- κ B pathways.

Mast cells lead to degranulation and generation of inflammatory mediators including histamine, chemokines, and cytokines under immunologic stimulation [50]. These mediators are important in the onset and development of AD [51]. Histamine provokes pruritus, which is one of the most prominent clinical features of AD [35]. Histamine is formed by HDC which is the catabolic enzyme of histamine synthesis [52]. In this study, TR significantly suppressed the expression and activity of HDC in activated HMC-1 cells. And TR significantly suppressed the level of serum histamine and scratching behavior in DNFB-induced AD murine model. The levels of IL-4 and IgE were significantly increased in serum from AD pediatric patients [53]. Th1-type IFN- γ is elevated in the chronic lesions, Th2-type cytokines such as IL-4 are elevated as well in lesions from AD patients [54]. In the lesional skin of patients with AD, the levels of TNF- α and IL-6 were increased compared to healthy individuals [55,56]. TARC is overproduced in the skin of patients with AD and attracts Th2 cells into the skin [57]. In this study, the levels of TSLP, IL-4, IFN- γ , IL-6, TNF- α , and TARC were also significantly suppressed by administration of TR in the AD murine model. Therefore, we can infer that TR would have an anti-AD effect by inhibiting histamine-induced scratching behavior and the expression of inflammatory cytokines and chemokine.

Activation of T cells is triggered through the simultaneous stimulation of the T

cell receptor/CD3 complexes and costimulatory receptors such as CD28, by antigen-presenting cells [58]. Purified splenic T cells were activated with anti-CD3/anti-CD28 antibodies [59]. In this study, TR significantly suppressed the production of IL-4, IFN- γ , and TNF- α from the anti-CD3/anti-CD28-stimulated splenocytes. When we stimulated splenocytes with anti-CD3/anti-CD28 antibodies, the production of TSLP was significantly increased. To our knowledge, this is the first report that stimulation with anti-CD3/anti-CD28 antibodies induces the production of TSLP in splenocytes. However, TR did not significantly suppress the production of TSLP from the stimulated splenocytes. The results nevertheless suggest that the anti-AD effect of TR is regulated at least partially through a suppressive action on splenocytes as well as mast cells.

It is noteworthy that TR suppressed the level of TSLP through the caspase-1 pathway from mast cells and the AD murine model. Furthermore, TR suppressed the levels of inflammatory cytokines, chemokine, and histamine in the AD murine model. These evidences illustrate that TR has the potential to be a therapeutic agent for AD aggravated by TSLP.

Acknowledgements

This research was supported by Naju city (2012) and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A1A2A10044645).

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Figure legends

Fig. 1. TR suppresses the level of TSLP from the activated HMC-1 cells. (a) HMC-1 cells were pretreated with TR (0.1, 1, and 10 μM) or BAPTA-AM (10 μM) for 20 min and then activated with PMACI. The kinetics of intracellular calcium was measured every 10 s for 500 s. Blank, inactivated cells; PMACI, PMACI-activated cells. (b) HMC-1 cells (4×10^5) were pretreated with TR (0.1, 1, and 10 μM) for 2 h and activated with PMACI for 7 h. The production of TSLP was analyzed with the ELISA. (c) HMC-1 cells (1×10^6) were pretreated with TR (0.1, 1, and 10 μM) for 2 h and activated with PMACI for 5 h. And mRNA expression of TSLP was analyzed with the real time-PCR analysis. (d) Cell viability was analyzed with an MTT assay. Each datum represents the mean $\pm$ SD of three independent experiments. $^{\#}P < 0.05$; significantly different from inactivated cells. $^{*}P < 0.05$; significantly different from PMACI-activated cells.

Fig. 2. TR suppresses the activation of Rip2/caspase-1/NF- κB in the PMACI-activated HMC-1 cells. (a) HMC-1 cells (5×10^6) were pretreated with TR (0.1, 1, and 10 μM) for 2 h and activated with PMACI for 1 h. The levels of Rip2 and caspase-1 were analyzed with Western blot analysis. (b) The relative intensities to actin were quantified by densitometry. (c) The caspase-1 activity was analyzed with a caspase-1 assay kit. (d) Catalytic activity of recombinant caspase-1 (0.5 units) was analyzed by tetrapeptide WEHD- $p\text{NA}$ (substrate) for indicated times. Blank, no substrate; Control, substrate-treated group; TR, substrate and TR-treated group. Each datum represents the mean $\pm$

SD of three independent experiments. [#]*P* < 0.05; significantly different from no substrate. **P* < 0.05; significantly different from substrate-treated group. (e) HMC-1 cells (5×10^6) were pretreated with TR for 2 h and activated with PMACI for 2 h. The activation of NF- κ B and phosphorylation of I κ B α were analyzed with Western blot analysis. NE, nuclear extract; CE, cytoplasmic extract. (f) The relative intensities to PARP or tubulin were quantified by densitometry. [#]*P* < 0.05; significantly different from inactivated cells. **P* < 0.05; significantly different from PMACI-activated cells.

Fig. 3. TR suppresses the level of IL-1 β from the activated HMC-1 cells. (a) HMC-1 cells (4×10^5) were pretreated with TR (0.1, 1, and 10 μ M) for 2 h and activated with PMACI for 7 h. The production of IL-1 β was analyzed with the ELISA. (b) HMC-1 cells (1×10^6) were pretreated with TR (0.1, 1, and 10 μ M) for 2 h and activated with PMACI for 5 h. And mRNA expression of IL-1 β was analyzed with the real time-PCR analysis. Each datum represents the mean $\pm$ SD of three independent experiments. [#]*P* < 0.05; significantly different from inactivated cells. **P* < 0.05; significantly different from PMACI-activated cells.

Fig. 4. TR suppresses the clinical symptoms in skin lesions of DNFB-induced AD murine model. The clinical features were observed 4 h after the last DNFB challenge. (a) Histological analysis of skin lesions were examined by H&E staining for inflammatory cells and epidermal thickness. E, epidermis. The inflammatory cells were indicated by arrows. Representative photomicrographs were examined at 400 $\times$ magnification. (b) The number of scratching behavior was measured at 4 h after the last

DNFB challenge for 10 min. DEX, dexamethasone. [#]*P* < 0.05; significantly different from vehicle group. ^{*}*P* < 0.05; significantly different from control group (DNFB). *n* = 5.

Fig. 5. TR suppresses the level of TSLP in skin lesions of DNFB-induced AD murine model. (a) The mRNA expression of TSLP was analyzed with RT-PCR analysis. The relative TSLP mRNA expression to GAPDH was quantified by densitometry. (b) The protein expression of TSLP from the skin lesion homogenate was analyzed with ELISA. (c) The caspase-1 activity from the skin lesion homogenate was analyzed with a caspase-1 assay kit. (d) The caspase-1⁺ (FITC) and c-Kit⁺ (PE) cells from DNFB-induced skin lesions were examined with a confocal laser-scanning microscope. Mast cells are identified as c-Kit⁺ cells. The merged image indicated the mast cells and caspase-1 colocalization. The caspase-1⁺c-Kit⁺ cells were indicated by arrows. Representative photomicrographs were examined at 120 × magnification. (Scale bar = 20 μm). DEX, dexamethasone. [#]*P* < 0.05; significantly different from vehicle group. ^{*}*P* < 0.05; significantly different from control group (DNFB). *n* = 5.

Fig. 6. TR suppresses the levels of AD-mediated factors in DNFB-induced AD murine model. (a) The mRNA expressions were analyzed with RT-PCR analysis. (b) The relative mRNA expressions to GAPDH were quantified by densitometry. (c) The protein expressions from the skin lesion homogenate were analyzed with ELISA. (d,e) The intensity of serum histamine was assayed as described in methods section. The levels of serum IgE and IL-4 were analyzed with ELISA. DEX, dexamethasone. [#]*P* < 0.05; significantly different from vehicle group. ^{*}*P* < 0.05; significantly different from control group (DNFB).

Fig 1

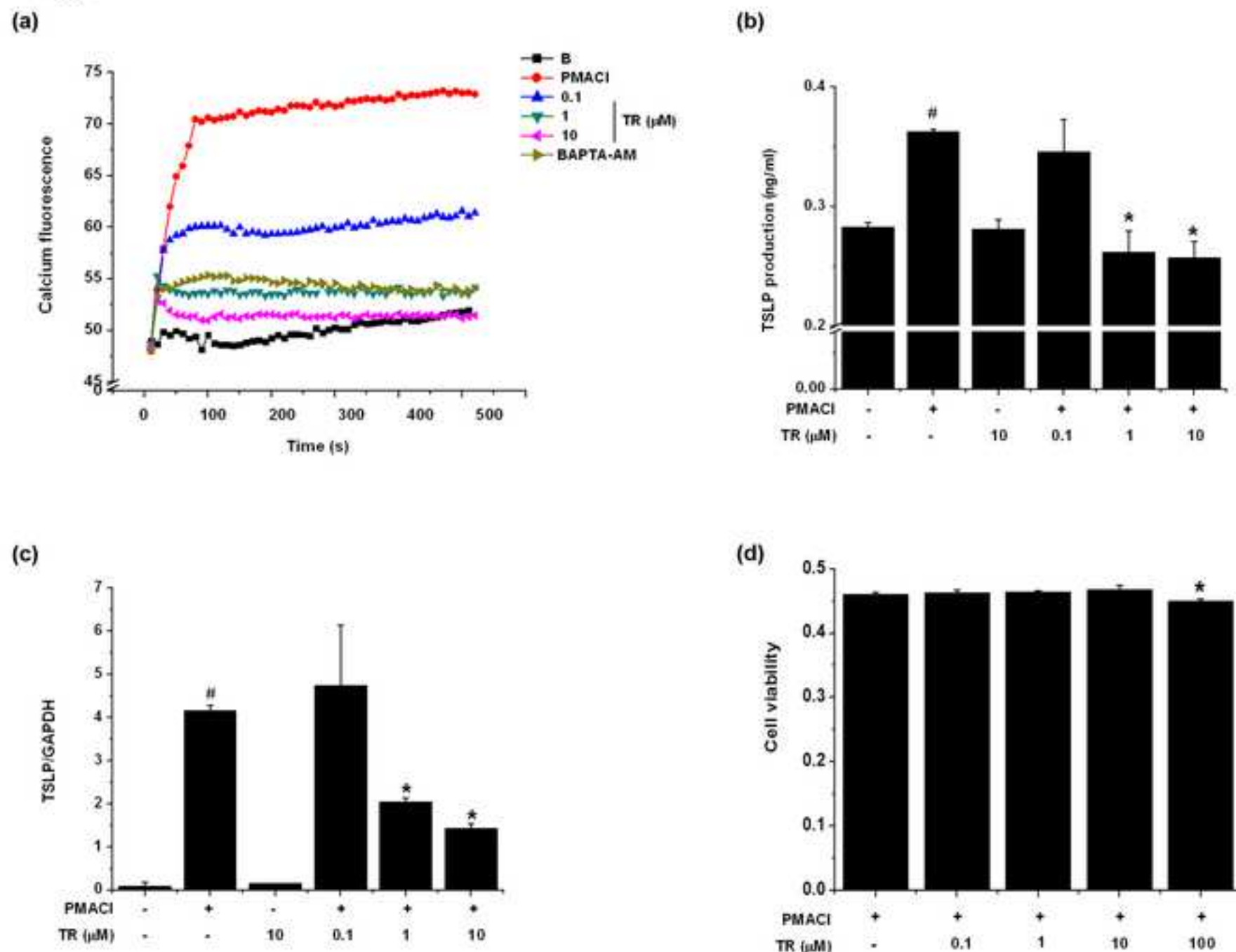
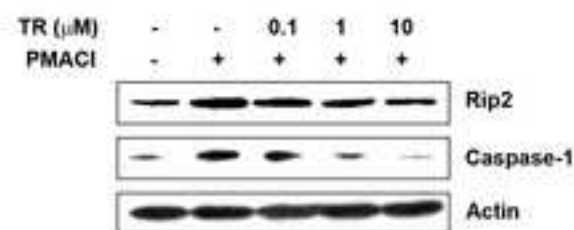
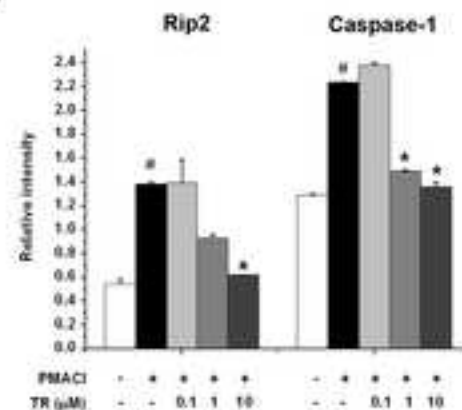


Fig 2

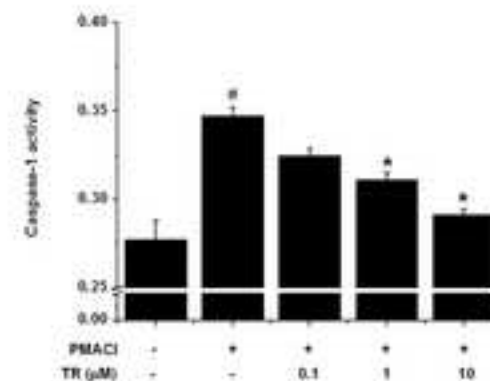
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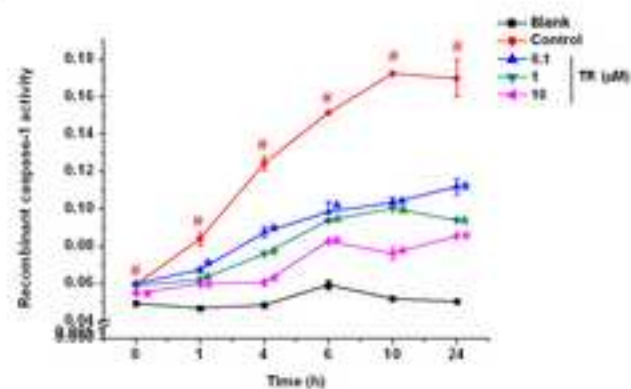
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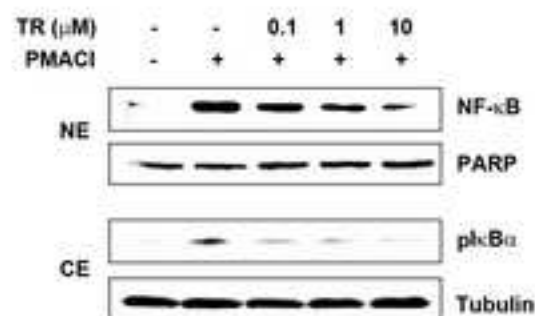
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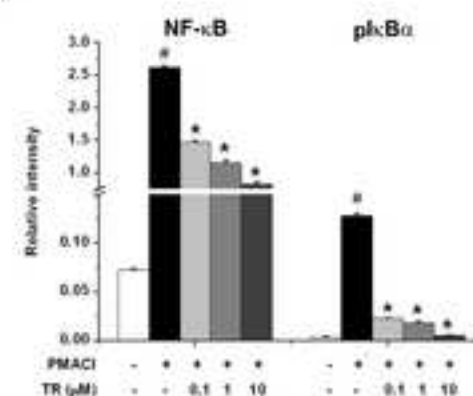
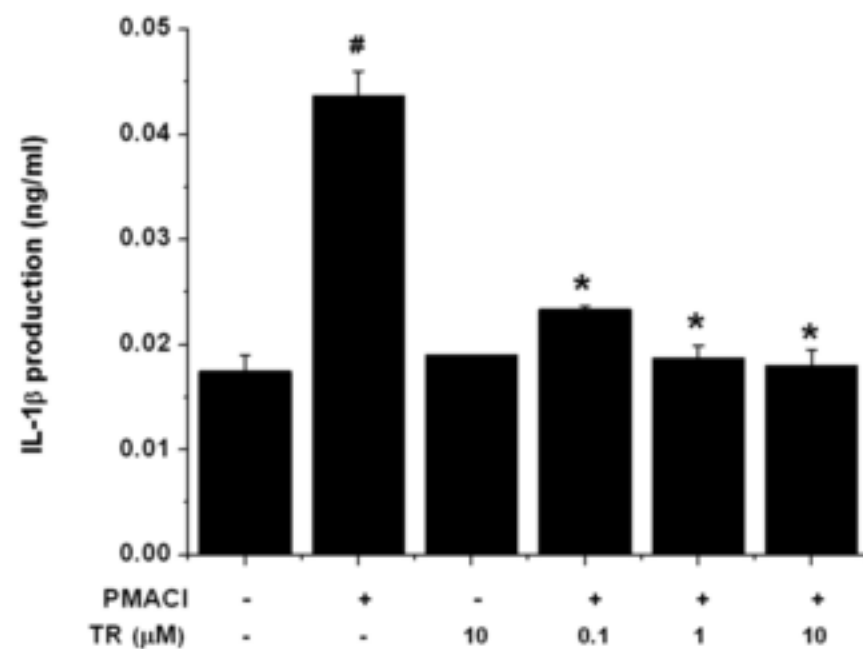


Fig 3
(a)



(b)

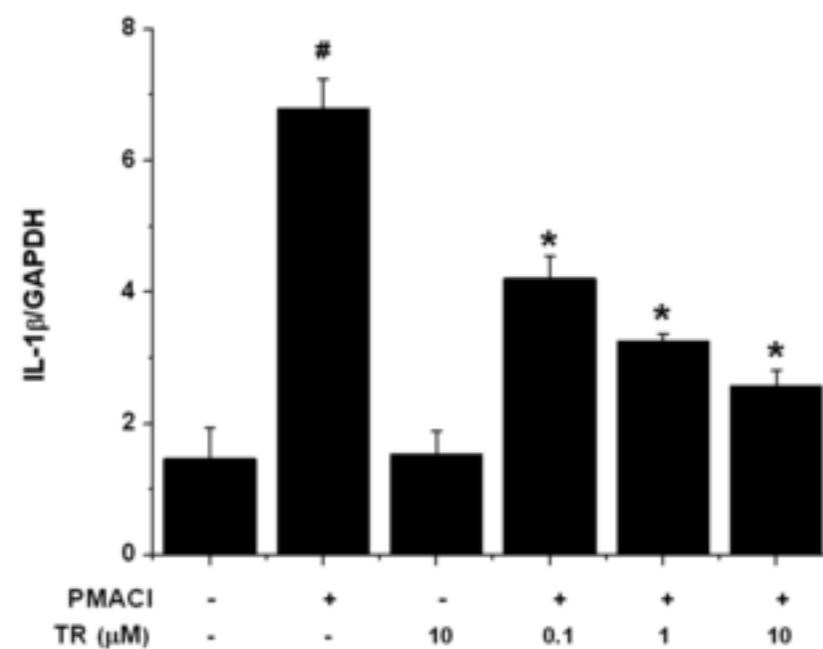
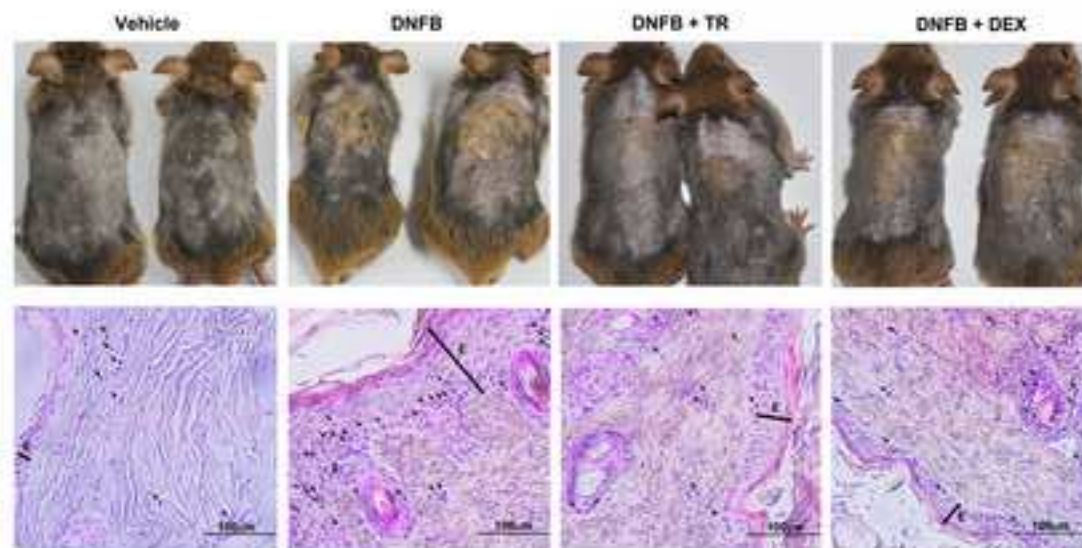


Fig 4
(a)



(b)

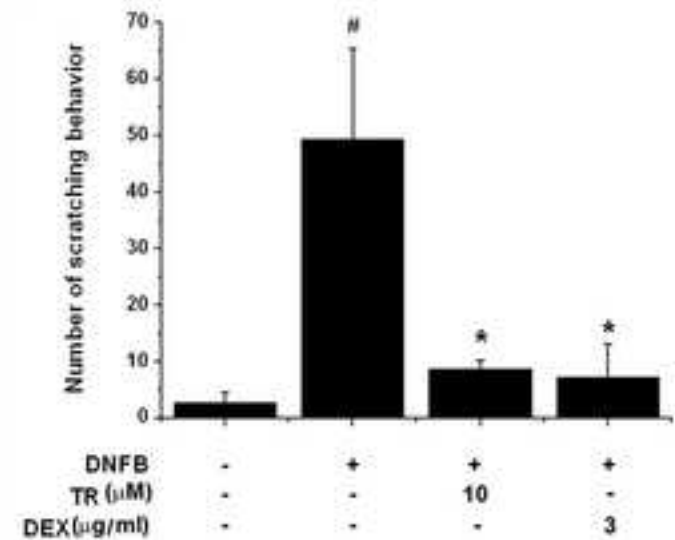


Fig 5

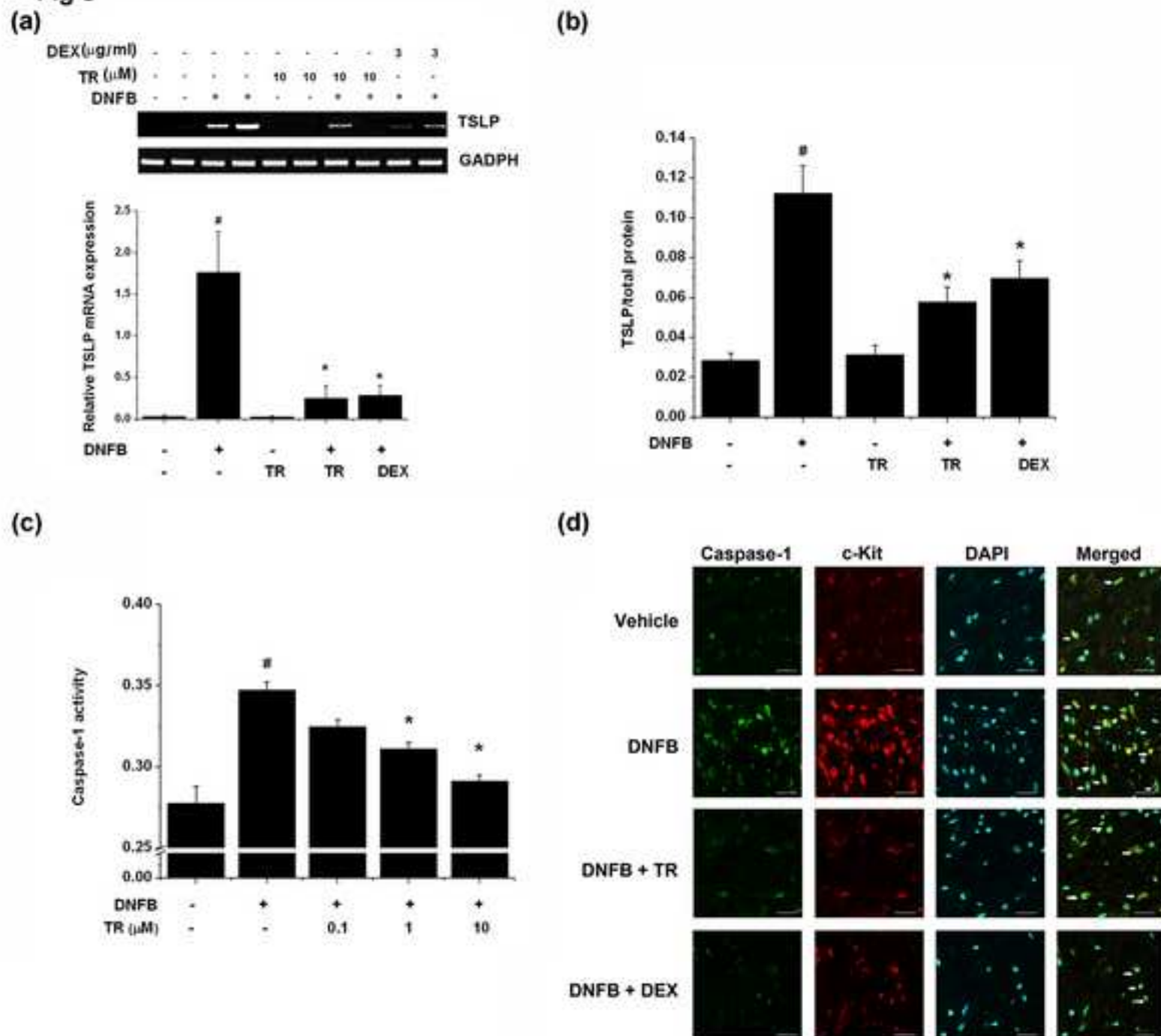
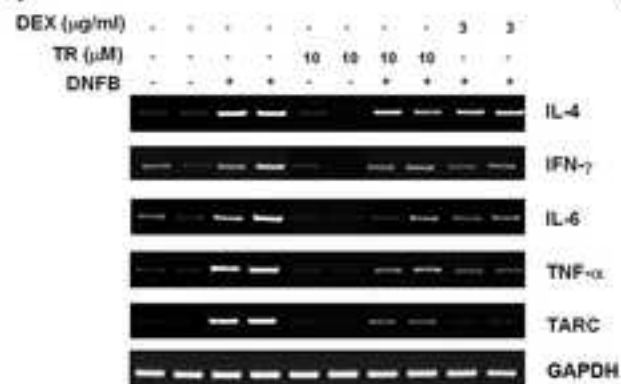
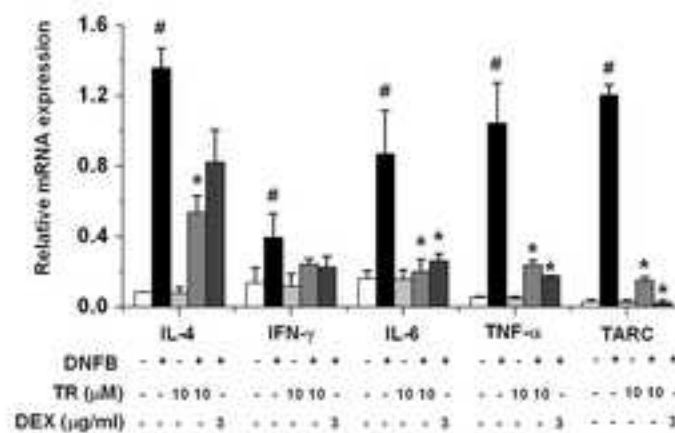


Fig 6

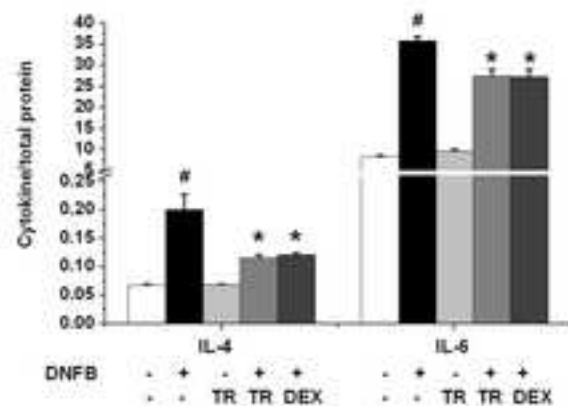
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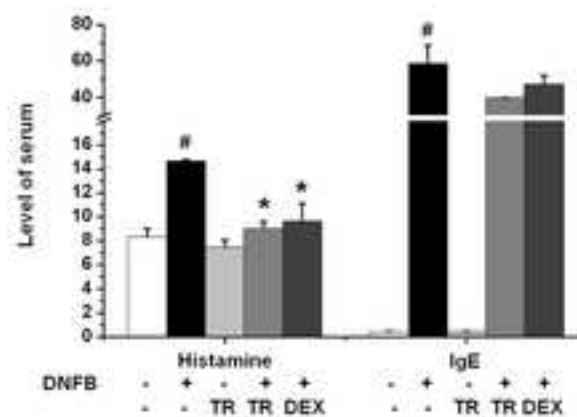
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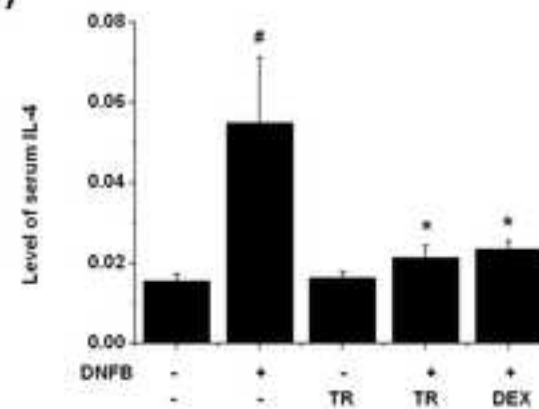
(c)



(d)



(e)



- We identified the regulatory effect of tryptanthrin (TR) on atopic dermatitis (AD).
- TR suppressed the level of TSLP through the caspase-1 pathway from mast cells.
- TR ameliorated clinical symptoms in AD model.
- TR inhibited the expressions of TSLP and caspase-1 in skin lesions of AD model.