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Research article

Histamine suppresses T helper 17 responses mediated by transforming growth factor-β1 in murine chronic allergic contact dermatitis

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Abstract: Allergic skin diseases are caused by the introduction of antigen into the skin. Repeated application of antigens prompts the development of atopic dermatitis (AD) and chronic allergic contact dermatitis (CACD). Histamine facilitates the development of chronic lesions in CACD. T helper (Th)17 cells are less prevalent in the chronic lesional skin compared to acute lesional skin in AD and CACD. The present experiment determined the effects of histamine in regulating Th17 in a murine model of CACD. CACD was induced by repeated epicutaneous challenge of 2,4,6-trinitro-1-chlorobenzene in *histidine decarboxylase* (HDC) (-/-) mice. Th17 response were analyzed by transforming growth factor (TGF)-β1, interleukin (IL)-17 and IL-22 levels in lesional skin. TGF-β1 was injected into the dermis of CACD-developing mice. Histamine H1 or H4 receptor antagonist were orally administrated in CACD-developing mice. IL-17 and IL-22 levels were lower in HDC (+/+) mice compared to HDC (-/-) mice. TGF-β1 levels were also lower in HDC (+/+) mice compared to HDC (-/-) mice. TGF-β1 injection increased IL-17 and IL-22 levels in the lesional skin of HDC (+/+) mice. Histamine H1 or H4 receptor antagonist administration also increased TGF-β1, IL-17 and IL-22 levels in the lesional skin of HDC (+/+) mice. In conclusion, histamine suppresses Th17 function in murine CACD. This effect was induced by the down-regulation of TGF-β1 through histamine H1 and H4 receptors.

Keywords: histamine; chronic allergic contact dermatitis; Th17; IL-17; IL-22; TGF-β1

1. Introduction

A single epicutaneous application of a contact sensitizing agent derives contact hypersensitivity responses in mice previously sensitized with the same agent. Contact hypersensitivity, acute dermatitis, is a delayed type and T helper (Th)1 dominant response. However, in many allergic skin diseases, allergic inflammations are caused by the chronic introduction of antigens into the skin. In patients with chronic allergic contact dermatitis (CACD), repeated exposure to antigens through the skin is thought to contribute to the development of eczematous lesions. Repeated application of antigens results in antigen-specific hypersensitivity responses from a delayed- to an early-type response and the accumulation of mast cells in the upper part of the dermis and elevation of serum IgE levels [1,2]. CACD induces a shift in cutaneous cytokine milieu from a Th1 to a Th2 profile [3]. Atopic dermatitis (AD), aggravated by chronic exposure to antigens, is a common and distinctive form of allergic skin diseases associated with eczematous lesions, early-type hypersensitivity and Th2 dominant responses, and increased IgE production in response to environmental allergens [4]. AD bears clinical, histological, and immunological similarities to CACD [5,6].

Histamine is a major mediator of allergic reactions and inflammation. High amounts of histamine are released during allergic and inflammatory disorders [7]. The pharmacological assessment of the in vivo effects of histamine had been observed by the use of histamine receptor antagonists. To overcome this limitation, histamine-deficient mice were produced by disrupting *histidine decarboxylase* (HDC) gene. Although histamine does not affect contact hypersensitivity responses, it facilitates the development of eczematous lesions in a murine model of CACD which uses HDC (-/-) mice [8,9]. Histamine produces IL-4 and IL-5 and promotes Th2 reaction in CACD [9]. Histamine H1 or/and H4 receptor antagonists inhibit the production of Th2 cytokines and ameliorates CACD, Th2 dominant disease [10,11].

Th17 cells, a distinct lineage of effector $CD4^+$ T cells, are characterized by their production of interleukin (IL)-17 and IL-22 [12] and are related to IL-4 production by Th2 cells [13]. The percentage of Th17 cells is increased in the peripheral blood of AD patients [14]. Increased IL-4 leads to decreased levels of IL-17 and IL-22 in CACD [15]. Therefore, histamine could be one of the important factors regulating Th17 responses in CACD developed by histamine. Since the relationship between histamine and Th17 responses had not been investigated in allergic dermatitis, I observed effects of histamine in regulating Th17 responses in a murine model of CACD using HDC (-/-) mice in the present study. Furthermore, the effects of histamine H1 or H4 receptor antagonist were investigated on Th17 responses in murine CACD model.

2. Materials and methods

2.1. Animals and agents

All experiments were performed in accordance with the guidelines for the care and use of experimental animals of the Animal Research Committee of Sagami Women's University. Histamine-deficient mice were generated by disrupting the HDC gene [16] and were kindly provided by Tohoku University. All experiments were performed using 9- to 12-week-old female C57BL/6 mice. The mice were housed with soft bedding, a 12-h light/dark cycle, and *ad libitum* access to food and water. 2,4,6-Trinitro-1-chlorobenzene (TNCB) was obtained from Sigma (St. Louis, MO, USA)

and dissolved in acetone to obtain a 1% solution. Mouse anti-transforming growth factor (TGF)-β1 and recombinant TGF-β1 (R & D Systems, Minneapolis, MN, USA) were diluted with 0.1 M sodium phosphate-buffered solution (PBS). H1 receptor antagonist olopatadine hydrochloride (olopatadine) or H4 receptor antagonist JNJ7777120 was kindly provided from Kyowa Hakko Kirin (Tokyo, Japan) or Johnson & Johnson Pharmaceutical Research & Development (San Diego, CA, USA), separately. Olopatadine or JNJ7777120 was suspended in 0.5% methyl cellulose or 20% hydroxypropyl-beta-cyclodextrin (Wako Pure Chemical Industries, Osaka, Japan) in distilled water, separately. For histological assessment, the optimal cutting temperature (OCT) embedding compound was obtained from Sakura FineTek (Tokyo, Japan) and anti-IL-17F antibody was obtained from Rockland (Gilbertsville, PA, USA).

2.2. Sensitization and challenge procedure

Repeated challenges (approximately 10 times) induce epidermal hyperplasia, accumulation of large numbers of mast cells and $CD4^+$ T cells beneath the epidermis, and elevated levels of IgE and Th2 cytokines [1,2,6,8,9,11]. Since these findings were observed in CACD and AD, sensitization and challenge procedure was carried out in accordance with Figure 1. HDC (-/-) mice and wild type mice were sensitized by applying 40 μl of 1% TNCB solution on the dorsal skin (day 0). Mice were challenged daily for 10 days using 20 μl of 1% TNCB solution on the same part of the skin day beginning on the 7^{th} day after sensitization up to 16 days. On day 17, the skin was collected 24 h after the final challenge.

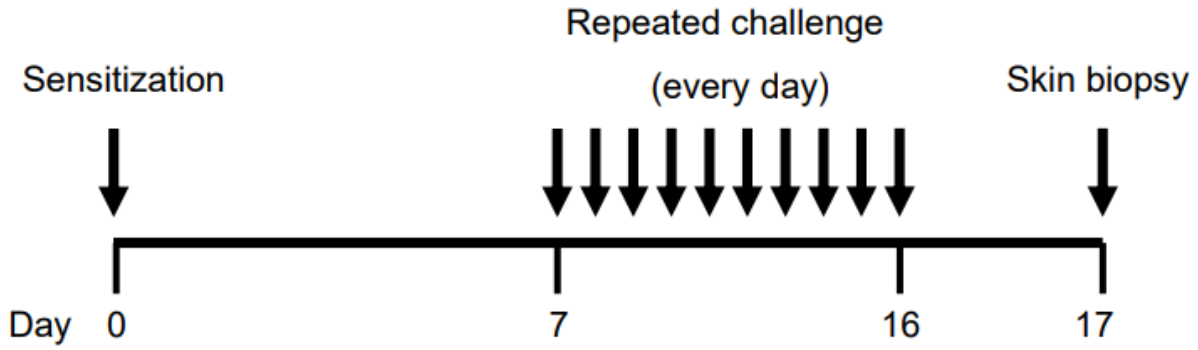

Figure 1. Experimental schedule of the chronic allergic contact dermatitis model. Mice were sensitized on day 0. Starting on day 7 after the sensitization, mice were challenged daily for 10 days on the same part of the skin for days 7–16. On day 17, 24 h after the final challenge, the skin was collected.

2.3. Agent injections

A hundred microliters of anti-TGF-β1 antibody (2.5 μg/ml) and recombinant TGF-β1 (0.25 μg/ml) were injected separately and daily into the dorsal dermis just before the TNCB challenge (days 7–16).

2.4. Histamine H1 receptor antagonist or H4 receptor antagonist administration

The H1 receptor antagonist olopatadine (10 mg/kg/day) or H4 receptor antagonist JNJ7777120 (100 mg/kg/day) was orally administered 30 min before each challenge to determine the effects of histamine H1 or H4 receptor on Th17 induction in CACD (days 7–16).

2.5. Histological assessment

The skin was biopsied and a part of the sample (4 mm in diameter) was fixed in 4% paraformaldehyde in 0.1 M PBS (pH 7.4) for 5 h and then immersed in 20% sucrose and embedded in OCT embedding compound. Frozen 5-μm sections were thoroughly rinsed and stained with hematoxylin-eosin and toluidine blue. Sections were then immunohistochemically stained with anti-IL17F antibody.

2.6. Measurement of cytokines

A skin sample (approximately 200 mg) was homogenized in 500 μl PBS solution (0.1 M) containing a protease inhibitor (CompleteTM; Roche, Mannheim, Germany) for 2 min using the Microtube Homogenizer (Kenis, Osaka, Japan) and centrifuged at 5000 × g for 5 min. Mouse ELISA kits (R & D Systems, Minneapolis, MN, USA) were used in accordance with the manufacturer's instruction to measure the concentration of IL-4, 17, 22 and 23 and TGF-β1 in the supernatant. Optical density at 450 nm was measured for each well in a Model 680 Microplate Reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

2.7. Statistical analysis

Values are presented as means ± standard deviation. Statistically significant difference between the two groups was estimated using the two-tailed Student's *t*-test. Differences were considered statistically significant if $p < 0.05$.

3. Results

3.1. Histamine suppresses Th17 activities in CACD

As reported in our previous study using TNCB ointment [9], repeated application of TNCB resulted in scaly erythema. Biopsies showed increased epidermal hyperplasia and epidermal thickness of HDC (+/+) mice was significantly larger than that of HDC (-/-) mice (Figures 2a, b, g). Remarkable dermal fibrosis with dense cell infiltration was observed in HDC (+/+) mice compared to HDC (-/-) mice (Figures 2a, b). Mast cells were more prevalent in the dermis of eczematous lesions in HDC (+/+) mice compared to HDC (-/-) mice, while IL-17 (+) cells were scarcer in HDC (+/+) mice compared to HDC (-/-) mice (Figures 2c–f). The number of mast cells in HDC (+/+) mice was significantly larger than that in HDC (-/-) mice (Figure 2h). On the other hand, the number of IL-17 (+) cells in HDC (+/+) mice was significantly smaller than that in HDC (-/-) mice (Figure 2i). IL-4 levels were significantly higher in HDC (+/+) mice compared to HDC (-/-) mice

(Figure 2j). IL-17 and IL-22 levels in the skin, cytokines produced by Th17 cells, were significantly lower in HDC (+/+) mice compared to HDC (-/-) mice (Figures 3a, b). Levels of TGF-β1, a cytokine inducer of Th17 cells, were also significantly lower in HDC (+/+) mice compared to HDC (-/-) mice (Figure 3c). On the other hand, levels of IL-23 levels, a cytokine establishing Th17 cells, did not differ between HDC (+/+) and HDC (-/-) mice (Figure 3d).

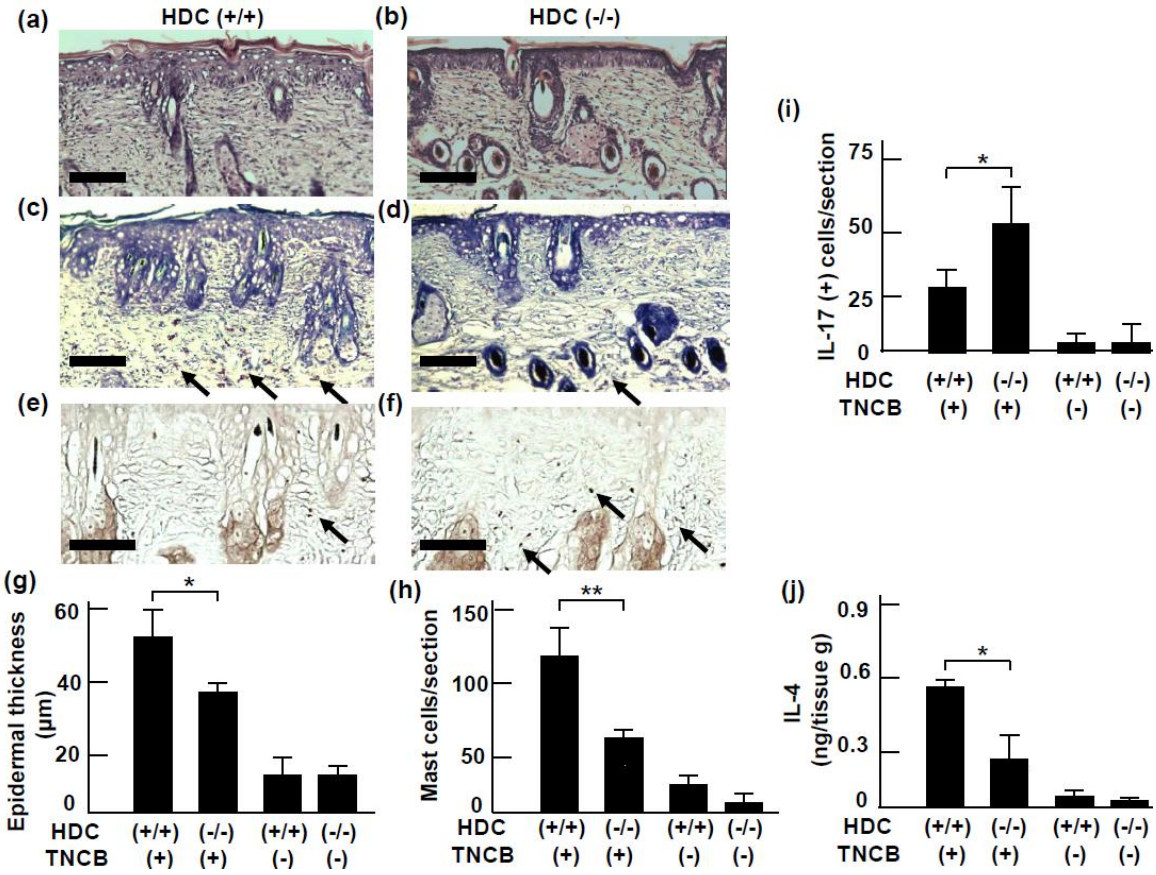

Figure 2. Histology, numbers of mast cells and IL-17 (+) cells, and IL-4 levels of HDC (+/+) and HDC (-/-) mice in chronic allergic contact dermatitis model. (a, b) Hematoxylin and eosin staining. (c, d) Toluidine blue staining. (e, f) IL-17 staining. (a–f) Scale bars: 50 μm. Arrows indicate toluidine blue or IL-17 positive cells. (a, c, e) HDC (+/+) mice. (b, d, f) HDC (-/-) mice. Original magnifications: ×100. (g) Epidermal thickness. (h) Numbers of mast cells. (i) Numbers of IL-17 (+) cells. (j) IL-4 levels in the skin. Description of the abscissa in each graph: HDC; histidine decarboxylase, TNCB; 2,4,6-trinitro-1-chlorobenzene. Data are expressed as mean ± SD (n = 6). *$p < 0.05$, **$p < 0.01$, n.s., not significant.

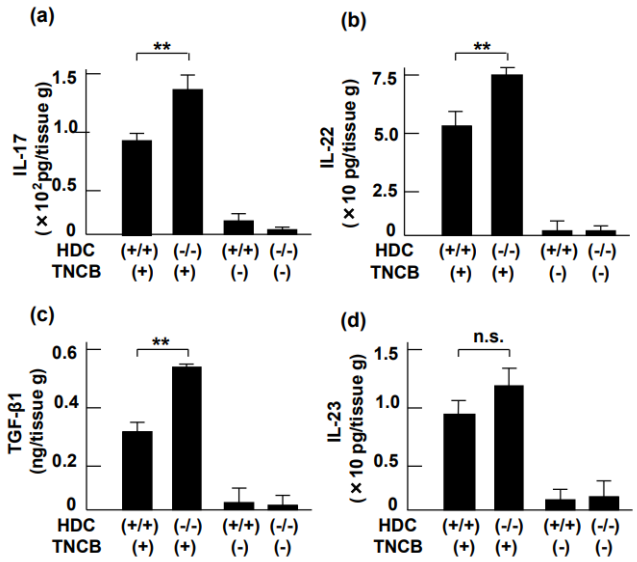

Figure 3. Th17-related cytokine levels of HDC (+/+) and HDC (-/-) mice in chronic allergic contact dermatitis model. (a) IL-17 levels in the skin. (b) IL-22 levels in the skin. (c) TGF-β1 levels in the skin. (d) IL-23 levels in the skin. Description of the abscissa in each graph: HDC; histidine decarboxylase, TNCB; 2,4,6-trinitro-1-chlorobenzene. Data are expressed as mean ± SD (n = 6). ** $p < 0.01$, n.s., not significant.

3.2. TGF-β1 administration induces the production of Th17 cytokines in CACD

Recombinant mouse TGF-β1 or mouse anti-TGF-β1 antibody was injected into the dorsal dermis just before each daily challenge of TNCB to assess the effects of TGF-β1 on Th17 in CACD (days 7–16). Injection of recombinant mouse TGF-β1 increased IL-17 and IL-22 levels in the eczematous lesions compared to solvent-injected mice (Figures 4a, b). On the contrary, these were decreased by injection of mouse anti-TGF-β1 antibody (Figures 4a, b).

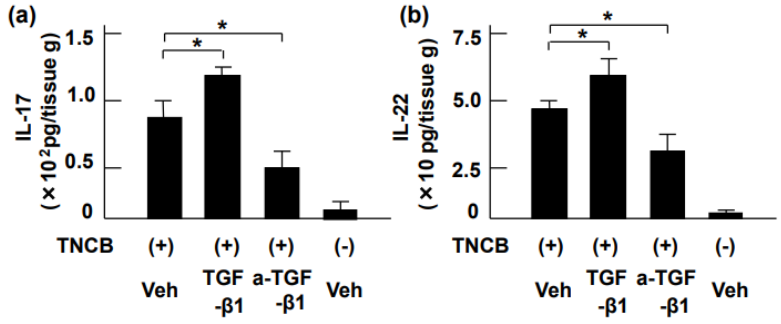

Figure 4. Effects of TGF-β1 and anti-TGF-β1 antibody on IL-17 and IL-22 induction in chronic allergic contact dermatitis model. TGF-β1 or anti-TGF-β1 antibody was injected daily just before the TNCB challenge. IL-17 (a) and IL-22 (b) levels in the skin. Description of the abscissa in each graph: TNCB; 2,4,6-trinitro-1-chlorobenzene, a-TGF-β1; anti-TGF-β1 antibody, Veh; vehicle injected. Data are expressed as mean ± SD (n = 5). * $p < 0.05$.

3.3. Histamine H1 or H4 receptor antagonist induces the production of Th17 cytokines in CACD

Olopatadine (histamine H1 receptor antagonist) or JNJ7777120 (histamine H4 receptor antagonist) was administrated 30 min before each daily challenge of TNCB to assess the effects of histamine receptors on Th17 in CACD (days 7–16). TGF-β1 levels in eczematous lesions were significantly increased by treatment with olopatadine or JNJ7777120 (Figure 5a). IL-17 and IL-22 levels were also significantly increased by treatment with olopatadine or JNJ7777120 (Figures 5b, c).

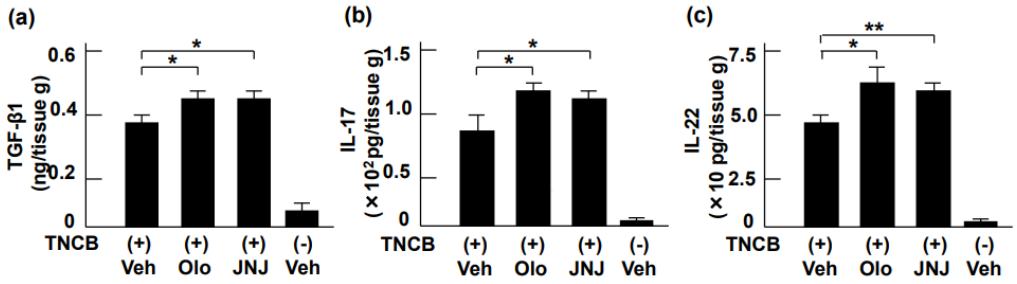

Figure 5. Effects of H1 and H4 receptor antagonist on IL-17 and IL-22 induction in chronic allergic contact dermatitis model. Histamine H1 or H4 receptor antagonist was orally administrated daily 30 min before the TNCB challenge. TGF-β1 (a), IL-17 (b) and IL-22 (c) levels in the skin. Description of the abscissa in each graph: TNCB; 2,4,6-trinitro-1-chlorobenzene, Olo; olopatadine, JNJ; JNJ7777120, Veh; vehicle administrated. Data are expressed as mean ± SD (n = 8). *$p < 0.05$, **$p < 0.01$.

4. Discussion

Allergic contact dermatitis is one of the most common skin diseases. Repeated applications of sensitizing agents have been reported to cause the evolution of the lesion into the chronic phase, leading to a model of CACD [5,17,18]. In HDC (-/-) mice, no plasma extravasation reaction was observed after a passive anaphylaxis test [19]. In contrast to immediate-type responses, contact hypersensitivity (delayed-type responses) showed no difference between HDC (+/+) and HDC (-/-) mice [9,19]. However, histamine was found to aggravate CACD eczematous lesions using this CACD model in HDC (-/-) mice [8]. In this CACD model, skin levels of Th2 cytokines (IL-4 and IL-5), serum levels of IgE and the numbers of mast cells and eosinophils increase in the presence of histamine [9]. These levels are controlled through H1 and H4 receptors [9,20]. Furthermore, histamine suppresses regulatory T cells mediated by TGF-β1 and develops Th2 responses in CACD lesions [21]. These studies confirm that histamine is an important factor in the development of CACD, Th2 dominant disease.

Th17 cells, a distinct lineage of effector $CD4^+$ cells, are characterized by their production of IL-17 and IL-22 [12]. IL-17 plays an important role in activating T cells in allergen-specific T-cell-mediated immune responses [22]. IL-17 is required for the sensitization phase of hapten-induced contact hypersensitivity responses [22]. The role of IL-17 is recognized in the elicitation phase of human allergic contact dermatitis [23]. The number of Th17 cells is increased in the peripheral blood of AD patients [14]. However, Th17 cells are less prevalent in the chronic than acute lesions of AD patients [14]. IL-17 and IL-22 levels are lower in the chronic eczematous lesions

of CACD compared to the lesions of contact hypersensitivity [15]. These observations suggest that the activity of Th17 may be suppressed in the chronic lesional skin of AD and CACD. Recent studies have demonstrated that Th2 responses have the ability to antagonize Th17 responses [24]. Topical application of acrylate gel enhances Th2 responses and downregulates Th17 responses [25]. A microRNA-210 inhibits Th2 differentiation but induces Th17 cell differentiation [26]. AD-mesenchymal stem cells show a down-regulation of Th2 cytokines, while Th17 cytokines are upregulated in AD-mesenchymal stem cells [27]. Absence of both IL-4 and IFN-gamma results in augmented Th17 differentiation [13]. The development of Th17 cells from naïve precursor cells is inhibited by IL-4 and IFN-gamma [28]. In the present study, Th2 responses induced by histamine may suppress Th17 responses.

TGF-β1 and IL-6 are both required for the induction of Th17 cells [28,29]. Histamine suppresses TGF-β1 levels of the lesional skin [21], while histamine promotes expression of IL-6 in murine CACD [9]. In the present study, histamine also suppressed TGF-β1 levels of the lesional skin. Administration of TGF-β1 increased IL-17 and IL-22 levels in lesional skin. Furthermore, H1 and H4 receptor antagonist increased TGF-β1, IL-17 and IL-22 levels in lesional skin. Therefore, histamine may suppress the induction of Th17 mediated by TGF-β1 in murine CACD.

5. Conclusion

Taken together, our study show that histamine suppresses Th17 function in murine chronic allergic contact dermatitis. This effect is induced by down-regulation of TGF-β1 through histamine H1 and H4 receptors.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

Reference

1. Kim H, Kim JR, Kang H, et al. (2014) 7,8,4'-Trihyroxyisoflavone attenuates DNCB-induced atopic dermatitis-like symptoms in NC/Nga mice. *PLoS One* 29: e104938.
2. Kitagaki H, Fujisawa S, Watanabe K, et al. (1995) Immediate-type hypersensitivity response followed by a late reaction is induced by repeated epicutaneous application of contact sensitizing agents in mice. *J Invest Dermatol* 105: 749–755.
3. Yamaura K, Shimada M, Ueno K (2011) Anthocyanins from bilberry (Vaccinium myrtillus L.) alleviate pruritus in a mouse model of chronic allergic contact dermatitis. *Pharmacognosy Res* 3: 173–177.
4. Boothe WD, Tarbox JA, Tarbox MB (2017) Atopic dermatitis: Pathophysiology. *Adv Exp Med Biol* 1027: 21–37.

5. Lin L, Xie M, Chen X, et al. (2018) Toll-like receptor 4 attenuates a murine model of atopic dermatitis through inhibition of langerin-positive DCs migration. *Exp Dermatol* 27: 1015–1022.
6. Liu L, Guo D, Liang Q, et al. (2015) The efficacy of sublingual immunotherapy with Dermatophagoides farinae vaccine in a murine atopic dermatitis model. *Clin Exp Allergy* 45: 815–822.
7. Stander S, Steinhoff M (2002) Pathophysiology of pruritus in atopic dermatitis: An overview. *Exp Dermatol* 11: 12–24.
8. Seike M, Takata T, Ikeda M, et al. (2005) Histamine helps development of eczematous lesions in experimental contact dermatitis in mice. *Arch Dermatol Res* 297: 68–74.
9. Seike M, Furuya K, Omura M, et al. (2010) Histamine H4 receptor antagonist ameliorates chronic allergic contact dermatitis induced by repeated challenge. *Allergy* 65: 319–326.
10. Matsushita A, Seike M, Okawa H, et al. (2012) Advantage of histamine H4 receptor antagonist usage with H1 receptor antagonist for the treatment of murine allergic contact dermatitis. *Exp Dermatol* 21: 714–715.
11. Ohsawa Y, Hirasawa N (2012) The antagonism of histamine H1 and H4 receptors ameliorates chronic allergic dermatitis via anti-pruritic and anti-inflammatory effects in NC/Nga mice. *Allergy* 67: 1014–1022.
12. Liang SC, Tan X, Luxenberg DP, et al. (2006) Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* 203: 2271–2279.
13. Yi T, Chen Y, Wang L, et al. (2009) Reciprocal differentiation and tissue-specific pathogenesis of Th1, Th2 and Th17 cells in graft-versus-host disease. *Blood* 114: 3101–3112.
14. Koga C, Kabashima K, Shiraishi N, et al. (2008) Possible pathogenic role of Th17 cells for atopic dermatitis. *J Invest Dermatol* 128: 2625–2630.
15. Matsushita A, Seike M, Hagiwara T, et al. (2014) Close relationship between T helper (Th)17 and Th2 response in murine allergic contact dermatitis. *Clin Exp Dermatol* 39: 924–931.
16. Ohtsu H, Tanaka S, Terui T, et al. (2001) Mice lacking histidine decarboxylase exhibit abnormal mast cells. *FEBS Lett* 502: 53–56.
17. Tamura T, Matsubara M, Takada C (2004) Effects of olopatadine hydrochloride, antihistamine drug, on skin inflammation induced by repeated topical application of oxazolone in mice. *Brit J Dermatol* 151: 1133–1142.
18. Kim JY, Jeong MS, Park MK, et al. (2014) Time-dependent progression from the acute to chronic phases in atopic dermatitis induced by epicutaneous allergen stimulation in NC/Nga mice. *Exp Dermatol* 23: 53–57.
19. Ohtsu H, Kuramasu K, Tanaka S, et al. (2002) Plasma extravasation induced by dietary supplemented histamine in histamine-free mice. *Eur J Immunol* 32: 1698–1708.
20. Hamada R, Seike M, Kamijima R, et al. (2006) Neuronal conditions of spinal cord in dermatitis are improved by olopatadine. *Eur J Pharmacol* 547: 45–51.
21. Tamaka K, Seike M, Hagiwara T, et al. (2015) Histamine suppresses regulatory T cells mediated by TGF-β in murine chronic contact dermatitis. *Exp Dermatol* 24: 280–284.
22. Nakae S, Komiyama Y, Nambu A, et al. (2002) Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity* 17: 375–387.

23. Zhao Y, Balato A, Fishelevich R, et al. (2009) Th17/Tc17 infiltration and associated cytokine gene expression in elicitation phase of allergic contact dermatitis. *Brit J Dermatol* 161: 1301–1306.
24. Kim D, McAlees JW, Bischoff LJ, et al. (2018) Combined administration of anti-IL-13 and anti-IL-17A at individually sub-therapeutic doses limits asthma-like symptoms in a mouse model of Th2/Th17 high asthma. *Clin Exp Allergy*, 24.
25. Bian R, Tang J, Hu L, et al. (2018) (E)-phenethyl 3-(3,5-dihydroxy-4-isopropylphenyl) acrylate gel improves DNFB-induced allergic contact hypersensitivity via regulating the balance of Th1/Th2/T17/Treg cell subsets. *Int Immunopharmacol* 65: 8–15.
26. Wu R, Zeng J, Yuan J, et al. (2018) MicroRNA-210 overexpression promotes psoriasis-like inflammation by inducing Th1 and Th17 cell differentiation. *J Clin Invest* 128: 2551–2568.
27. Orciani M, Campanati A, Caffarini M, et al. (2017) T helper (Th)1, Th17 and Th2 imbalance in mesenchymal stem cells of adult patients with atopic dermatitis: at the origin of the problem. *Brit J Dermatol* 176: 1569–1576.
28. Harrington LE, Hatton RD, Mangan PR, et al. (2005) Interleukin 17-producing $CD4^+$ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineage. *Nat Immunol* 6: 1123–1132.
29. Mangan PR, Harrington LE, O'Quinn DB, et al. (2006) Transforming growth factor-beta induces development of the T_H17 lineage. *Nature* 441: 231–234.