

Forum Minireview

**Analysis of the Mechanism for the Development of Allergic Skin Inflammation and the Application for Its Treatment:
Establishment of a Modified Allergic Dermatitis Model in Mouse Ear Lobes by Application of 12-*O*-Tetradecanoyl Phorbol 13-Acetate:
Putative Involvement of Thymic Stromal Lymphopoietin and Roles of Histamine**

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Abstract. We established a novel allergic dermatitis model in mouse ear lobes in which antigen-nonspecific inflammation was induced by painting 12-*O*-tetradecanoylphorbol 13-acetate (TPA) between sensitization and challenge with picryl chloride (PiCl). This model has an advantage for analyzing atopic dermatitis-like inflammation within a short period. Analysis of the time course changes in the PiCl-induced swelling showed that the allergic inflammation was shifted from a delayed-type response to a biphasic response consisting of a weak immediate-phase response and a late-phase response by painting with TPA. The application of TPA increased the PiCl-induced infiltration of eosinophils and mast cells at the inflammatory site and shifted the cytokine milieu from Th1 to Th2. The expression of the Th2-inducing cytokine thymic stromal lymphopoietin (TSLP) mRNA was also increased by TPA. These findings suggested that the induction of antigen-nonspecific inflammation by TPA before the antigen challenge enhanced the Th2 response and modified the PiCl-induced delayed type-hypersensitivity. Using this model, we clarified that histamine plays significant roles in the early-phase swelling via H₁ receptors and the late-phase swelling via H₃/H₄ receptors. Thus, we suggested the usefulness of the combined treatment with an H₁ antagonist and an H₄ antagonist for the suppression of atopic dermatitis.

Keywords: allergic dermatitis, histamine, picryl chloride, 12-*O*-tetradecanoylphorbol 13-acetate, thymic stromal lymphopoietin, allergic inflammation

Introduction

Atopic dermatitis is an allergic inflammatory disease characterized by intense pruritus, chronic eczematous

plaques, and relapsing inflammation induced by repeated exposure to the antigen. In the inflamed skin, the infiltration of eosinophils, the number of mast cells, and Th2-type immune responses are generally increased (1, 2). At present, there are several animal models of dermatitis with atopic dermatitis-like skin lesions. For example, repeated epicutaneous exposure to chemical compounds such as 2,4,6-trinitro-1-chlorobenzene (3 – 5) and para-phenylenediamine (6) or dust mite allergen (7) resulted in chronic contact hypersensitivity. NC/Nga mice have

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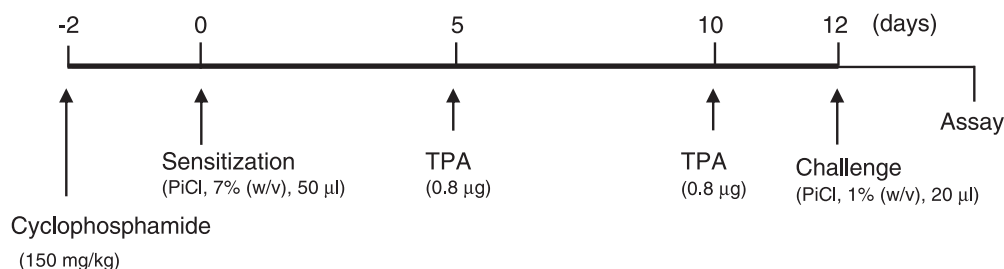


Fig. 1. Induction of TPA-modified allergic dermatitis in mouse ear lobes.

also been used as a model of atopic dermatitis (8–10). The contact hypersensitivity induced by contact-sensitizing chemicals such as picryl chloride (PiCl) involved Th1-dominant inflammation (3, 5). However, repeated application leads to responses different from those induced by a single challenge. Repeated application of the antigen shifted the cytokine milieu from Th1 to Th2, resulting in increased infiltration of eosinophils and mast cells, and the induction of immediate- and late-phase responses (3–6). Antigen-nonspecific inflammation, such as infection and scratching, worsens atopic dermatitis. Grabbe et al. (11) reported that antigen-nonspecific irritant applied with a small amount of the antigen enhanced the antigen-induced response. To clarify whether antigen-nonspecific inflammation induced before antigen challenge affects the antigen-induced immune response, we established a novel contact dermatitis model in mouse ear lobes in which antigen-nonspecific inflammation was induced by painting 12-*O*-tetradecanoylphorbol 13-acetate (TPA) between sensitization and challenge with PiCl (12). Using this modified contact dermatitis model, we analyzed the shift to Th2-dominant inflammation and roles of thymic stromal lymphopoietin (TSLP) and histamine.

Establishment of a modified allergic dermatitis model in mouse ear lobes by application with TPA

To induce blood eosinophilia, cyclophosphamide was administered subcutaneously to male BALB/c mice (6–8 weeks of age; Charles River Japan, Inc., Kanagawa) at a dose of 150 mg/kg as described by Satoh et al. (13). Two days later, mice were sensitized with 50 µl of a 7% (w/v) PiCl (Nacalai Tesque, Inc., Kyoto) solution (3:1 in acetone : ethanol) or the vehicle by painting it on the right ear lobe (day 0). Thereafter, 20 µl of a 0.04 µg/µl TPA (Sigma-Aldrich, St. Louis, MO, USA) solution (3:1 in acetone : ethanol) was applied to the same site twice (days 5 and 10) to induce antigen-nonspecific inflammation. On day 12, mice were challenged with 20 µl of a 1% (w/v) PiCl solution or the vehicle by painting it on the right ear lobe (12) (Fig. 1). Ear thickness was

measured with a dial thickness gauge (Peacock; Ozaki MFG Co., Tokyo) at the indicated time. The ear lobe tissues were then obtained by using a punch (diameter 5.2 mm) and weighed. For the determination of the level of mRNA, the tissue samples were frozen in liquid nitrogen and stored at –80°C. The levels of mRNA for interferon (IFN)-γ, interleukin (IL)-4, TSLP, histidine decarboxylase (HDC), thymus and activation-regulated chemokine (TARC), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were determined by RT-PCR. For histological analysis, tissue specimens were immediately fixed in 10% neutral buffered formalin and embedded in paraffin. Sections, 4-µm thickness, were cut and stained with a Luxol-fast blue (EM Science, Cherry Hill, NJ, USA) solution for the detection of eosinophils or with a 0.05% (w/v) toluidine blue (EM Science) solution for the detection of mast cells. The numbers of eosinophils and mast cells were counted and expressed as the number of cells in the range of 0.25 mm (12). The number of eosinophils in blood was determined by Giemsa staining.

We found that this model showed features similar to those observed in atopic dermatitis: crust formation, epidermal hyperplasia, and a vigorous infiltration of leukocytes including eosinophils (12). Analysis of the time course changes in the PiCl-induced swelling showed that the allergic inflammation was shifted from a delayed-type response to a biphasic response consisting of a weak immediate-phase response and a late-phase response by painting with TPA (Fig. 2). The number of mast cells in ear lobes was also increased by the treatment with TPA to about 1.8-fold. The challenge with PiCl on day 12 increased both the levels of mRNA for IFN-γ and IL-4 (Fig. 3, open columns). The application of TPA on days 5 and 10 decreased the level of IFN-γ mRNA, but increased that of IL-4 mRNA (Fig. 3, hatched columns), indicating that the application of TPA induced a shift in the cytokine milieu from a Th1- to a Th2-type profile (12). Thus, this model will be a suitable model for studying the mechanisms by which the antigen-nonspecific inflammation worsens allergic dermatitis.

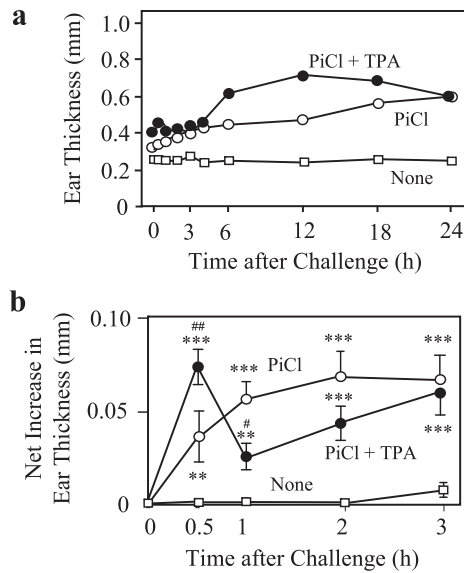


Fig. 2. Effects of TPA application on the changes in ear thickness with time in the mice exposed to PiCl. Mice were treated as described in the text. Each point is a mean $\pm$ S.E.M. (shown by vertical lines) from 4 mice. a: Ear lobe thickness was measured from 0 to 24 h after the PiCl challenge. Some of the S.E.M. values were too small to depict on the graph, i.e., they are within the symbols. b: Ear thickness of the mouse before the challenge was subtracted from the data in (a) and indicated as the net increase in ear thickness. ** $P < 0.01$, *** $P < 0.001$ vs. the corresponding control group (None); # $P < 0.05$, ## $P < 0.01$ vs. the corresponding PiCl group. Reproduced with permission from Int Arch Allergy Immunol (Ref. 12).

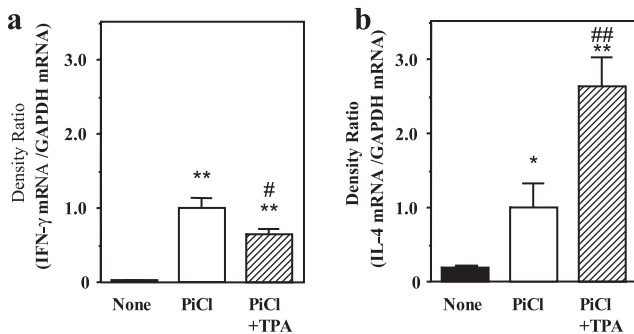


Fig. 3. Change of cytokine milieu in the ear lobe tissue. The ear lobe tissue was excised 8 h after the challenge with PiCl, and the total RNA was extracted. The levels of mRNA for IFN- γ and IL-4 were determined by RT-PCR, and the density ratios of IFN- γ mRNA (a) and IL-4 mRNA (b) to GAPDH mRNA were calculated. The mean density ratio of each cytokine in the "PiCl" group was set to 1.0. The values are the means from five mice with S.E.M. shown by vertical bars. Statistical significance: * $P < 0.05$, ** $P < 0.01$ vs. None; # $P < 0.05$, ## $P < 0.01$ vs. PiCl group. Modified with permission from Int Arch Allergy Immunol (Ref. 12).

Putative involvement of TSLP

The precise mechanism for the change from Th1-dominant to Th2-dominant inflammation still remains

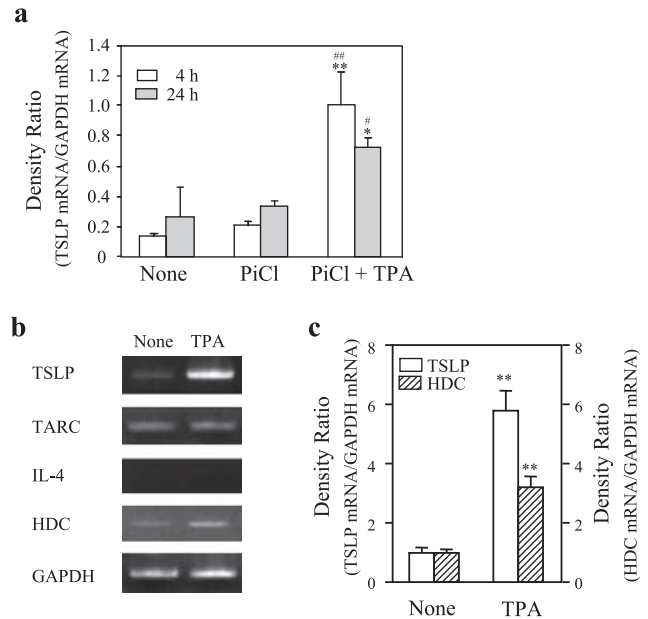


Fig. 4. Levels of mRNA for TSLP in the ear lobe tissue. At 4 and 24 h after the application of TPA on day 5, total RNA was extracted from the excised ear lobe tissue and the mRNA for TSLP was detected by RT-PCR. a: The density ratio of TSLP mRNA to GAPDH mRNA was calculated. The mean density ratio of the "PiCl + TPA" group 4 h after the TPA application was set to 1.0. The values are means from three to five mice with S.E.M. shown by vertical bars. Statistical significance: * $P < 0.05$, ** $P < 0.01$ vs. None; # $P < 0.05$, ## $P < 0.01$ vs. PiCl group at the corresponding time point. b and c: TPA or the vehicle was applied to the ear lobe of untreated mice. At 4 h after the application of TPA, total RNA was extracted from the excised ear lobe tissue and the mRNAs for TSLP, TARC, IL-4, HDC, and GAPDH were detected by RT-PCR. The density ratios of TSLP mRNA and HDC mRNA to GAPDH mRNA were calculated (c). The mean density ratio of untreated group was set to 1.0. The values are means from four mice with S.E.M. shown by vertical bars. Statistical significance: ** $P < 0.01$ vs. corresponding None. Modified with permission from Int Arch Allergy Immunol (Ref. 12).

to be clarified. TSLP has recently been reported to be implicated in the pathogenesis of atopic dermatitis. The main source of TSLP is epithelial cells including keratinocytes (14). TSLP activates CD11c⁺ dendritic cells and induces an expansion of the CD4⁺ T cell population (15). Because skin-specific overexpression of TSLP (16) resulted in an atopic dermatitis-like phenotype, TSLP might be one of the inducers for the Th2-dominant immune response in atopic dermatitis. However, it has not been determined whether TSLP is expressed in the skin when the shift of cytokine milieu is induced in the contact dermatitis model. In our model, the application of TPA to the ear lobe of the PiCl-sensitized mice markedly increased the level of TSLP mRNA at 4 h (Fig. 4a). The level was still significantly higher at 24 h (Fig. 4a). TPA treatment without PiCl

sensitization also increased the level of mRNA for TSLP at 4 h but not for other Th2 inducers TARC and IL-4 (Fig. 4: b and c). The level of mRNA for HDC at 4 h was also increased by TPA (Fig. 4: b and c). Thus TPA-induced production of TSLP might be one of the probable mechanisms for the change in the immune balance to Th2-dominant. It is reported that TSLP has an activity to induce production of TARC, a chemokine for CD4⁺ T cells (15), in dendritic cells and IL-4, a Th2 cytokine, in CD4⁺ T cells (17). However, 4 h after the TPA application, mRNA levels for TARC and IL-4 did not increase. Further study is necessary to clarify the involvement of TSLP in the enhancement of Th2 responses in this model.

Roles of histamine

Histamine, which is produced by HDC, plays significant roles in immediate-type allergic responses. Histamine also affects functions of immune and inflammatory cells. For example, histamine induces the production of TARC (18) and decreases the production of IL-12 in dendritic cells (19), resulting in the induction of Th2-dominated responses. In addition, histamine has an activity to recruit mast cells and eosinophils via H₄ receptors, which are expressed on eosinophils (20) and mast cells (21). In our model, the application of TPA significantly increased the number of mast cells (12) and the level of HDC mRNA (Fig. 4: b and c), suggesting that histamine plays a more significant role compared with that in the delayed-type hypersensitivity model induced by PiCl alone. This was also supported by the finding that the challenge with PiCl on the TPA-treated ear lobe induced an increase in the thickness in the immediate-phase, which was suppressed by pyrilamine (Fig. 5a). In contrast, the swelling 12 h after the PiCl challenge was more potently inhibited by thioperamide than by pyrilamine (Fig. 5b). In our model, the treatment with cyclophosphamide and TPA increased the number of eosinophils in the inflamed tissue (12), suggesting that a part of the late-phase response in this model might be elicited by eosinophils. We found that thioperamide inhibited the eosinophil infiltration consistent with the inhibition of the ear swelling in the late-phase (12). The histamine-induced chemotaxis of eosinophils is inhibited by the specific H₄ antagonist JNJ777120 (22); and in H₄ receptor-deficient mice, the infiltration of eosinophils in allergic airway inflammation is less extensive than that in wild-type mice (22). We also found that the infiltration of eosinophils was less in HDC-deficient mice than that in wild-type mice, suggesting that histamine plays a role in the infiltration of eosinophils, probably through H₄ receptors, in allergic

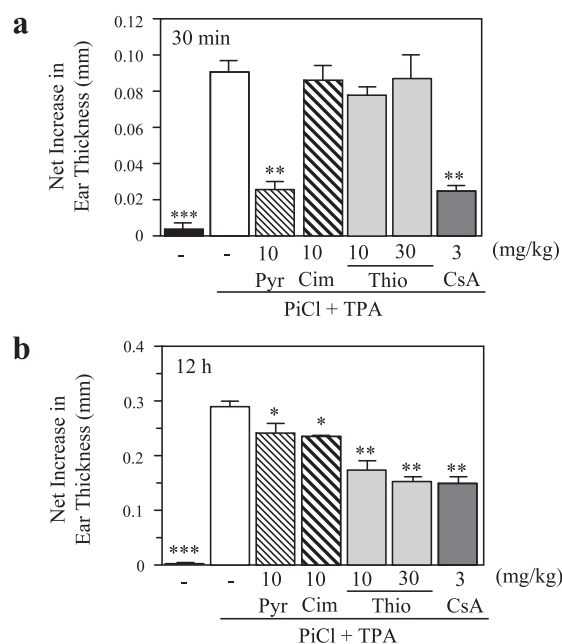


Fig. 5. Effects of histamine-receptor antagonists on the ear swelling in TPA-modified allergic dermatitis. On day 12, mice were intravenously administered the histamine-receptor antagonist pyrilamine (Pyr), cimetidine (Cim), or thioperamide (Thio); the immunosuppressor cyclosporin A (CsA); or the vehicle. Thirty minutes later, mice were challenged with a 1% (w/v) PiCl solution (PiCl + TPA) or vehicle (closed column). Ear thickness was measured before and at 30 min (a) and 12 h (b) after the challenge. The ear thickness of the mouse before the challenge was subtracted from the data. The values are the means from four mice with S.E.M. shown by vertical bars. Statistical significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. PiCl + TPA control. Reproduced with permission from Int Arch Allergy Immunol (Ref. 12).

dermatitis in mice. In contrast, although histamine is reported to recruit mast cells via H₄ receptors (21), there was no statistically significant difference in the increase in the number of mast cells between our model and that in HDC-deficient mice. This finding suggested that histamine does not participate in mast cell infiltration.

Cyclosporin A and tacrolimus inhibit both immediate- and delayed-type responses in mice (23). These drugs inhibit the production of cytokines (24) and the antigen-induced degranulation of mast cells and basophils (25, 26) and thereby suppress allergic dermatitis (23, 27). In our model, we confirmed that cyclosporin A inhibits the swelling of the ear lobe in both the immediate-phase and late-phase (Fig. 5 and Ref. 12). Furthermore, we found that the combined treatment with pyrilamine and thioperamide inhibited the swelling of the ear lobe, being as strong as cyclosporin A (12). These findings suggest that the combination of an H₁ antagonist with an H₄ antagonist might be a new strategy for the suppression of atopic dermatitis.

Table 1. Summary of the effect of anti-histamines and cyclosporine A on ear swelling in the TPA-modified allergic dermatitis model

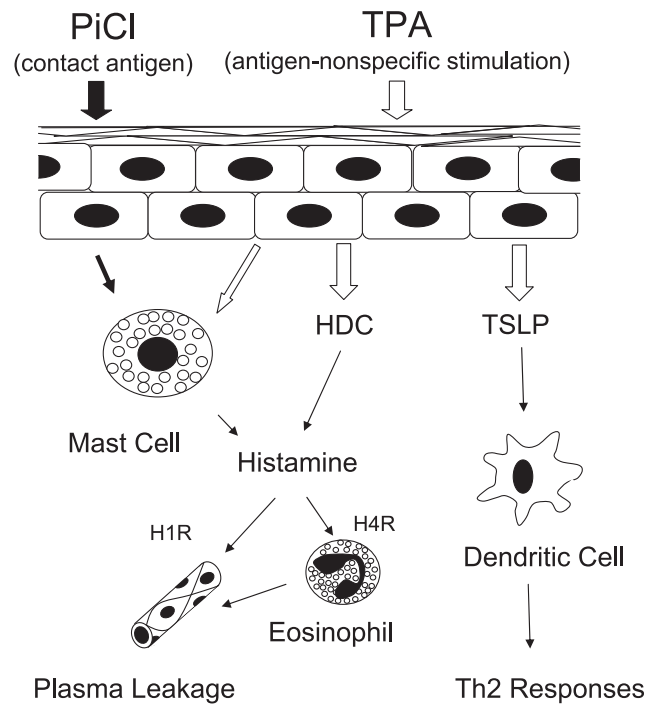
Treatment	Dose (mg/kg)	Ear swelling	
		30 min	12 h
Pyrilamine	10	↓↓↓	↓
Cimetidine	10	—	↓
Thioperamide	10	—	↓↓
Pyrilamine	3		
+ Thioperamide	10	↓↓↓	↓↓
Cyclosporin A	3	↓↓↓	↓↓

Conclusion

We established a novel model for atopic dermatitis. In this model, we found that the induction of the antigen-nonspecific inflammation by TPA before the antigen challenge enhanced Th2 responses and modified the PiCl-induced delayed-type hypersensitivity (Figs. 2 and 3). TPA increased the TSLP-mRNA level in the inflammatory site (Fig. 4). In addition, our findings indicated that histamine is involved in the infiltration of eosinophils and ear swelling in the late-phase via the H₄ receptor as well as the swelling in the immediate-phase via the H₁ receptor (Fig. 5). Thus, TSLP and histamine might be involved in the TPA-induced changes of contact dermatitis. Finally, we suggested the usefulness of the combined treatment with an H₁ antagonist and an H₄ antagonist for the suppression of atopic dermatitis. Pharmacological features of this TPA-modified allergic dermatitis model are summarized in Table 1. Proposed mechanisms for how histamine and TSLP are involved in the induction of the TPA-modified atopic dermatitis model are summarized in Fig. 6.

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**Fig. 6.** Putative roles of TSLP and histamine in TPA-modified allergic dermatitis.

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