

Original article

Histamine H₄ receptor antagonist ameliorates chronic allergic contact dermatitis induced by repeated challenge

Background: The present study observed effects of the histamine H₄ receptor on chronic allergic contact dermatitis induced by repeated challenge in mice.

Methods: Acute contact dermatitis was induced by single epicutaneous challenge of 2,4,6-trinitro-1-chlorobenzene (TNCB) to the ear. Chronic allergic contact dermatitis was developed by repeated epicutaneous challenge using TNCB on the dorsal back skin. H₄ receptor antagonist JNJ777120 was administered to wild-type mice, while H₄ receptor agonist 4-methylhistamine was administered to histidine decarboxylase (HDC) (–/–) mice that synthesized no histamine.

Results: HDC (–/–) mice did not differ phenotypically from HDC (+/+) mice, and H₄ receptor antagonist/agonist did not have clinical effects in terms of acute contact dermatitis reactions. H₄ receptor antagonist ameliorated skin eczematous lesions induced by repeated TNCB challenge in HDC (+/+) mice. On the contrary, H₄ receptor agonist exacerbated skin lesions exclusively in HDC (–/–) mice. Application of H₄ receptor agonist induced migration of mast cells and eosinophils in skin lesions, and H₄ receptor antagonist suppressed these changes. H₄ receptor was immunohistochemically detected on mast cells in eczematous lesions. Levels of interleukin (IL)-4, -5, and -6 in lesions were decreased, whereas levels of interferon- γ and IL-12 were increased by H₄ receptor antagonistic activity. Serum Immunoglobulin E levels rapidly increased with repeated challenge, but decreased with H₄ receptor antagonist.

Conclusion: Because chronic allergic contact dermatitis is developed by H₄ receptor stimulation, H₄ receptor antagonists might represent new candidate drugs for treating chronic allergic contact dermatitis.

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The histamine H₄ receptor has recently been cloned in human, mouse, rat and guinea pig (1, 2). H₄ receptor is expressed mainly in leukocytes and mast cells (1). Histamine induces chemotaxis of eosinophils through the H₄ receptor (3) and rapidly induces shape changes in eosinophils while at the same time enhancing their chemotactic response to chemokines through H₄ receptor (3, 4). H₄ receptor also mediates histamine-induced chemotaxis of mast cells in mouse (5, 6). Histamine-induced migration of mast cells is also observed *in vivo*, with histamine inhalation inducing an increase in the number of sub-epithelial mast cells in the trachea of mice (6). One of the main functions of H₄ receptor in eosinophils and mast cells is thus chemotaxis. H₄ receptor function has a role in the secretion of interleukin (IL)-16 from CD8⁺ T cells. IL-16 acts as a chemoattractant for CD4⁺ T cells. These findings of chemotaxis and IL-16 production indicate that H₄ receptor antagonists might be useful for treating allergic and inflammatory diseases.

Conversely, histamine downregulates human monocyte CCL2 production through H₄ receptor (7). Histamine and H₄ receptor agonists downregulate CCL2 and IL-12

expression on monocyte-derived human inflammatory dendritic epidermal cells (8). These findings suggest that instead of H₄ receptor antagonists, H₄ receptor agonists might have more beneficial effects in controlling inflammation. Given this context, we decided to clarify the activity of H₄ receptor in chronic allergic contact dermatitis.

Contact dermatitis is a commonly observed skin disease. Repeated application of various haptens induces chronic allergic lesions in the skin, and this method is used to model chronic allergic contact dermatitis in mouse (9, 10). Chronic allergic contact dermatitis shows eczematous lesions into which mast cells and eosinophils are infiltrating (11). Histamine facilitates the development of eczematous lesions in a murine model of chronic allergic contact dermatitis through H₁ receptor (11), while H₁ receptor antagonist diminishes the number of infiltrating cells and levels of cytokines in eczematous lesions (12). However, the signal through H₁ receptor could not explain all histamine activity, because H₁ receptor antagonist does not completely suppress development of eczematous lesions in wild-type mice, while H₁ receptor

agonist does not fully develop these lesions in histamine-deficient mice (11). Those results suggest that the other types of histamine receptor could be involved in the development of eczematous lesions. The present experiment observed the effects of histamine on chronic allergic contact dermatitis through H_4 receptor.

Materials and methods

Animals

Histamine-deficient mice were generated by disrupting the histidine decarboxylase (HDC) gene (13). All experiments were performed using 10- to 12-week-old female C57BL/6 mice. Mice were housed with soft bedding, a 12-h light/dark cycle, and *ad libitum* access to food and water. Experiments were undertaken following the guidelines for the care and use of experimental animals of the Animal Research Committee of Sagami Women's University.

Agents

2,4,6-Trinitro-1-chlorobenzene (TNCB) was obtained from Sigma Chemical Co. (St. Louis, MO), dissolved in acetone to 1% solutions and used for sensitization and challenge. H_4 receptor agonist 4-methylhistamine was purchased from Tocris Cookson Inc. (Ellisville, MO, USA) and dissolved in Phosphate Buffered Saline (PBS) (pH 7.4) for intradermal administration. H_4 receptor antagonist JNJ777120 was kindly presented from Johnson & Johnson Pharmaceutical Research & Development (San Diego, CA, USA), dissolved in a vehicle of 20% hydroxypropyl- β -cyclodextrin/water and orally administered at a volume of 10 ml/kg.

Sensitization and challenge procedure

Single epicutaneous challenge, a delayed-type hypersensitivity. Single challenge procedure is explained in Fig. 1A. Mice were sensitized by painting with 20 μ l of 1% TNCB solution to the right ear (day 0), and 20 μ l of 1% TNCB solution was applied to that sensitized right ear or nonapplied left ear 7 days (day 7) after the sensitization. One day after the challenge, a typical delayed-type hypersensitivity was observed. JNJ777120 was orally administered in HDC (+/+) mice, and 200 μ l of 4-methylhistamine was intradermally injected in HDC (-/-) mice 30 min before the challenge. Ear thickness was measured with Digimatic Micrometer (Mitutoyo Corporation, Kanagawa, Japan) 24 h after the challenge (day 8) under the ether anesthesia (9). Ear tissue was histologically examined following the procedure explained in 'Histological assessment' section.

Repeated epicutaneous challenge. Repeated challenge procedure is explained in Fig. 2A. Mice were challenged every day using 20 μ l of 1% TNCB solution on the dorsal back skin for 10 days, starting from 7th day after the sensitization (days 7–16). JNJ777120 and 4-methylhistamine administered 30 min before those challenges. The numbers of infiltrating cells were counted and levels of cytokines in eczematous lesions were measured in the skin at day 17.

Histological assessment. A part of skin biopsies were fixed in 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4) for 5 h, immersed in 20% sucrose and embedded in Optimal Cutting Temperature (OCT) compound (Sakura Finetech Co., Ltd.,

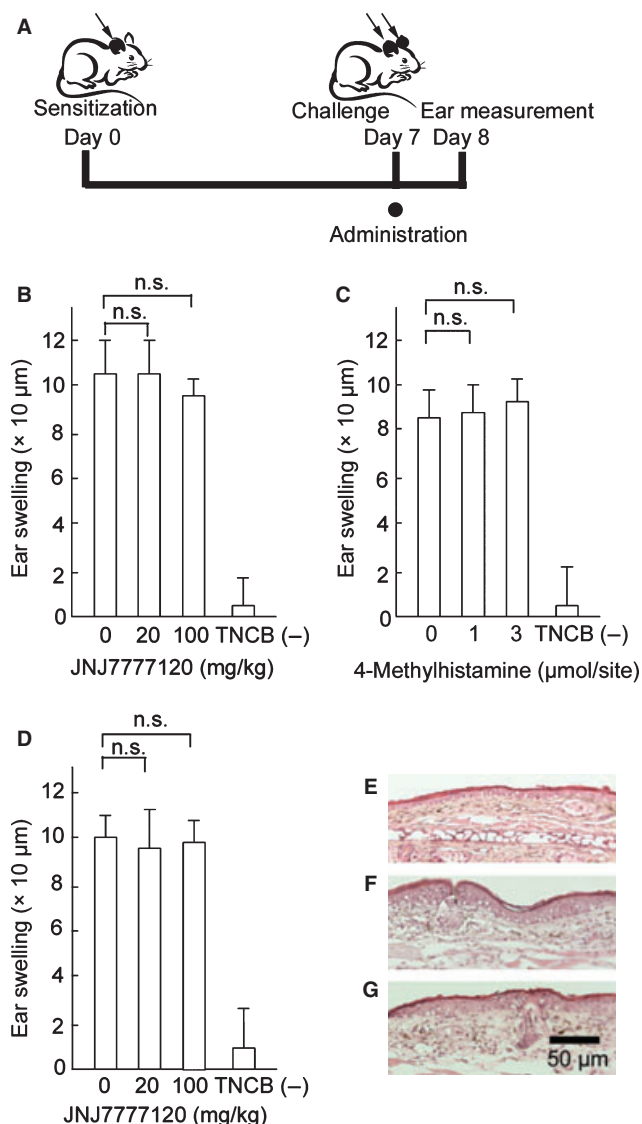


Figure 1. Single epicutaneous challenge. (A) Mice were sensitized on the right ears by epicutaneous application of 2,4,6-trinitro-1-chlorobenzene (TNCB) (day 0). Seven days after the sensitization (day 7), the original sensitized right or the non-sensitized left ears of these mice were challenged with TNCB. Ear thicknesses were measured 24 h after the challenge (day 8). JNJ777120 was administered in histidine decarboxylase (HDC) (+/+) mice, and 4-methylhistamine was performed in HDC (-/-) mice 30 min before the challenge. (B) Right ear swelling of HDC (+/+) mice. (C) Right ear swelling of HDC (-/-) mice. (D) Left ear swelling of HDC (-/-) mice. Data are expressed as mean $\pm$ SD ($n = 8$). n.s., not significant. (E–G) Histology of ear skins in HDC (+/+) mice. (E) TNCB-untreated. (F) TNCB-treated. (G) JNJ777120 and TNCB-treated. Hematoxylin and eosin staining.

Tokyo, Japan). Frozen sections of 5- μ m thickness were thoroughly rinsed and stained with hematoxylin-eosin, toluidine blue, berberine sulfate (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and EoProbe eosinophil staining kit (BioFX Laboratories, Inc.,

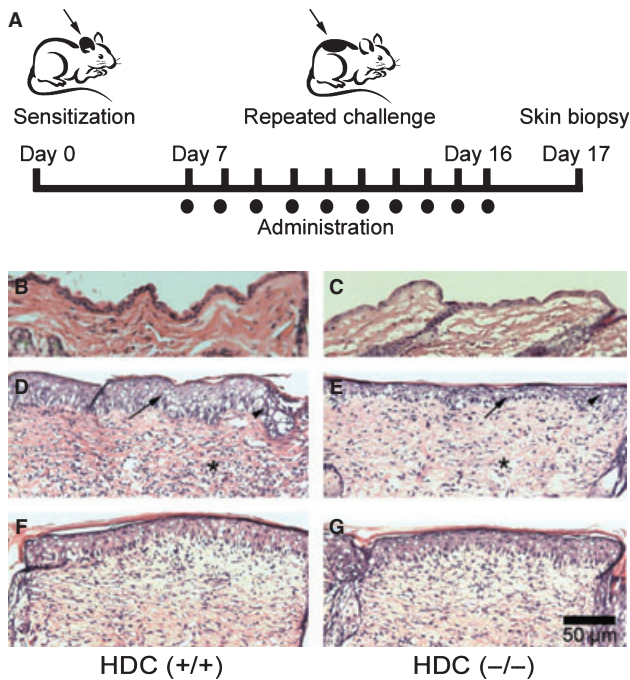


Figure 2. Repeated epicutaneous challenge. (A) Mice were sensitized using an application of 2,4,6-trinitro-1-chlorobenzene (TNCB) (day 0). Seven days after the sensitization, mice were challenged repeatedly using TNCB on the dorsal skin once a day for 10 days (day 7–16), and skin biopsies were performed a day after these repeated challenge (day 17). JNJ777120 was administered in histidine decarboxylase (HDC) (+/+) mice, and 4-methylhistamine was performed in HDC (-/-) mice everyday during repeated challenge 30 min before the challenge. JNJ777120 or 4-methylhistamine was administrated ten times as total. (B, C) TNCB-untreated. (D, E) TNCB-treated. (F) JNJ777120 and TNCB-treated. (G) 4-Methylhistamine and TNCB-treated. (B, D, F) HDC (+/+) mice. (C, E, G) HDC (-/-) mice. Hematoxylin and eosin staining.

Owings Mills, MD, USA) (14). Number of eosinophils or mast cells infiltrating in the skin were counted with hematoxylin-eosin staining and toluidine blue staining, respectively. Sections were immunohistochemically stained after anti-H₄ receptor antibody (10 µg/ml, Alpha Diagnostic International, San Antonio, TX, USA) treatment and by anti-IgE antibody treatment (10 µg/ml, Bethyl Laboratories, Inc., Montgomery, TX, USA).

Measurement of cytokines. A skin sample (approximately 100 mg) was homogenized in 200 µl PBS solution containing protease inhibitor (Complete™; Roche Diagnostics, Mannheim, Germany) for 1 min using Microtube Homogenizer (Kenis Ltd., Osaka, Japan) and centrifuged at 5000 *g* for 20 min. To measure the concentrations of interferon (IFN)-γ, IL-4, -5, -6 and -12 in the supernatant, Mouse ELISA kits (Ray Biotech, Inc., Norcross, GA, USA) was used in accordance with the instructions of the manufacturer. Optical density at 450 nm for each well was measured in a Model 680 Microplate Reader (Bio-Rad Laboratories, Hercules, CA, USA).

Measurement of serum IgE. Concentration of serum IgE was measured using Mouse IgE ELISA kit (Shibayagi Inc., Gunma, Japan) in accordance with the instructions of the manufacturer.

Statistical analysis

Values are presented as means ± standard error. Statistical significance between two groups was estimated using the two-tailed Student's *t*-test. Differences are statistically significant at *P* < 0.05.

Results

Ear-swelling response, a typical delayed-type hypersensitivity

Ear swelling in HDC (-/-) mice was not significantly different from that in HDC (+/+) mice in the single challenge procedure (Fig. 1B,C). H₄ receptor antagonist JNJ777120 did not influence ear swelling in HDC (+/+) mice (Fig. 1B). Likewise, H₄ receptor agonist did not influence ear swelling in HDC (-/-) mice (Fig. 1C). When the left ear was challenged instead of the sensitized right ear, ear swellings were not so different from swelling responses in the sensitized right ear, and again H₄ receptor antagonist did not influence the swelling (Fig. 1D). Inter cellular edema in the epidermis and infiltration of dermal cells including a small number of mast cells and eosinophils were observed with single challenge, and these changes were not markedly influenced by H₄ receptor antagonist (Fig. 1E–G). These findings suggest that H₄ receptor is not strongly involved in this ear swelling response, nor in mast cell and eosinophil infiltrating responses with typical delayed-type hypersensitivity.

Histamine facilitates development of eczematous lesions induced by repeated challenge through H₄ receptor

No marked histological differences were noted between control skin from HDC (+/+) and HDC (-/-) mice (Fig. 2B,C). Repeated challenge with TNCB resulted in the development of scaly erythema with hair loss. Biopsies showed marked epidermal hyperplasia (arrow) with intercellular edema (arrowhead) and intense dermal cell infiltration (asterisk) in HDC (+/+) mice (Fig. 2D). Conversely, moderate epidermal hyperplasia (arrow) with focal epidermal intercellular edema (arrowhead) and moderate dermal cell infiltration (asterisk) were observed in HDC (-/-) mice (Fig. 2E). H₄ receptor antagonist JNJ777120 was administered in HDC (+/+) mice to assess the effects of suppressing H₄ receptor function on the development of eczematous lesions induced by repeated challenge. Eczematous lesions were ameliorated in the presence of JNJ777120 in HDC (+/+) mice (Fig. 2F). H₄ receptor agonist 4-methylhistamine was administered in HDC (-/-) mice to assess the effects of H₄ receptor function on the development of eczematous lesions induced by repeated challenge. Eczematous lesions were aggravated in the presence of 4-methylhistamine in HDC (-/-) mice (Fig. 2G). Therefore, histamine facilitated development of eczematous lesions through H₄ receptor.

Infiltration of mast cells and eosinophils is mediated by H_4 receptor Mast cells (Fig. 3A,B) and eosinophils (Fig. 3C,D) had infiltrated into eczematous lesions induced by repeated challenge. H_4 receptor was expressed on IgE-positive cells in eczematous lesions (Fig. 3E–G). Cells stained by anti- H_4 receptor antibody were not immunohistochemically recognized in control skin from HDC (+/+) (Fig. 3H) or HDC (–/–) mice (data not shown), although mast cells were observed in these lesions. Administration of 100 mg/kg of JNJ777120 significantly decreased the number of mast cells and eosinophils in eczematous lesions of HDC (+/+) mice (Fig. 3I,J). Conversely, 1 and 3 μ mol of injected 4-methylhistamine significantly increased numbers of mast cells and eosinophils in eczematous lesions of HDC (–/–) mice (Fig. 3K,L). Numbers of mast cells in HDC (+/+) mice exceeded those in HDC (–/–) mice in both control skin and eczematous lesions (Fig. 3I,J). Eosinophils were not recognized in normal skin from HDC (+/+) and HDC (–/–) mice (Fig. 3K,L). These

data illustrate that infiltration by mast cells and eosinophils is under the control of H_4 receptor in this eczematous lesion model.

Productions of Th2 cytokines are increased by H_4 receptor activation

Levels of IL-4, -5, and -6 in the skin of both HDC (+/+) and HDC (–/–) mice were increased by repeated challenge with TNCB to produce eczematous lesions. Levels of IL-4 and -5, particularly IL-4, in eczematous lesions from HDC (+/+) mice were significantly decreased by treatment with 100 mg/kg of JNJ777120, while levels in HDC (–/–) mice were significantly increased by treatment with 3 μ mol of 4-methylhistamine (Fig. 4A–D). IL-6 levels in eczematous lesions of HDC (+/+) mice were significantly decreased by treatment with 100 mg/kg of JNJ777120, while levels in eczematous lesions of HDC (–/–) mice were not greatly increased by treatment with 4-methylhistamine (Fig. 4E,F).

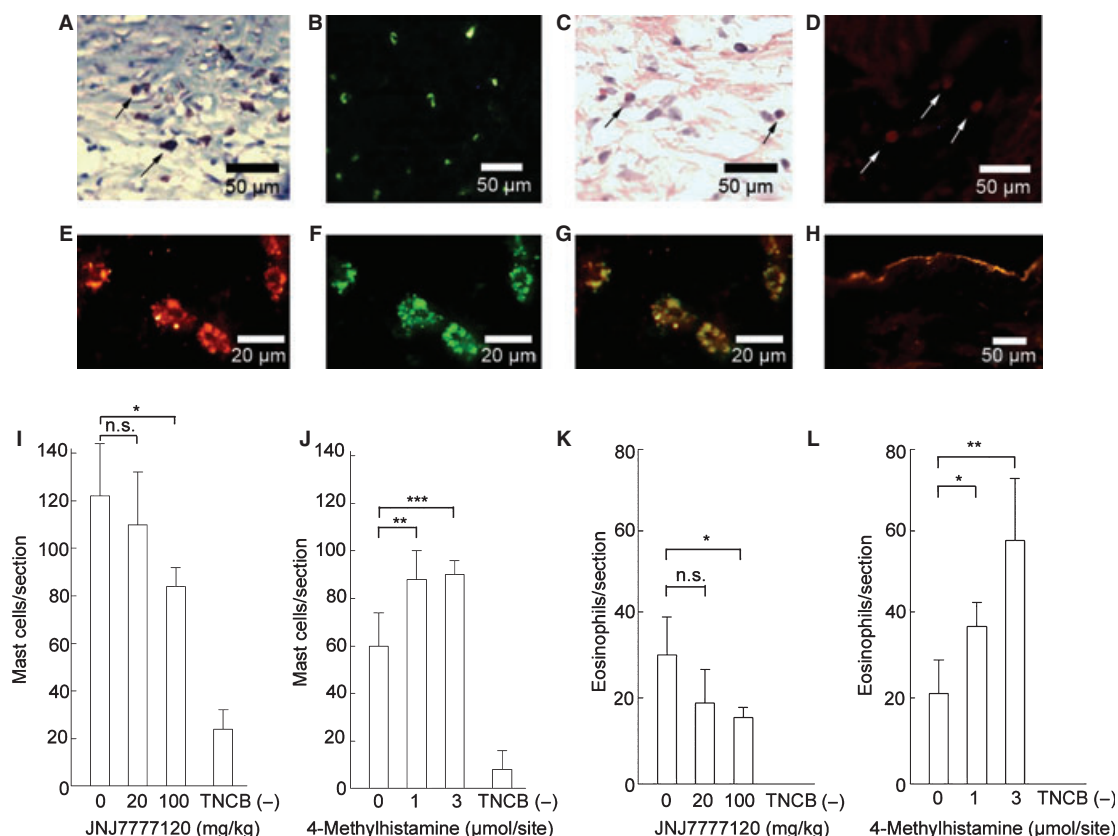


Figure 3. Mast cell and eosinophil infiltration. The procedure for the application of 2,4,6-trinitro-1-chlorobenzene (TNCB), JNJ777120, and 4-methylhistamine was the same as described in Fig. 2A. (A) Toluidine blue staining. (B) Berberine sulfate staining. (C) Hematoxylin and eosin staining. (D) Staining by EoProbe eosinophil staining kit. (E) H_4 receptor staining. (F) IgE staining. (G) Merged image. These histological analyses (A–G) have been performed using the frozen sections of histidine decarboxylase (HDC) (+/+) mice skin after repeated application of TNCB. (H) H_4 receptor staining of TNCB-untreated HDC (+/+) mouse skin. Arrows in (A) indicate toluidine blue positive cells, in (C) indicate eosine-stained cells, and in (D) indicate EoProbe-stained cells. Numbers of mast cells (I, J) and eosinophils (K, L) under the effect of JNJ777120 in HDC (+/+) mice (I, K) and under the effect of 4-methylhistamine in HDC (–/–) mice (J, L). Data are expressed as mean $\pm$ SD ($n = 8$). n.s., not significant. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

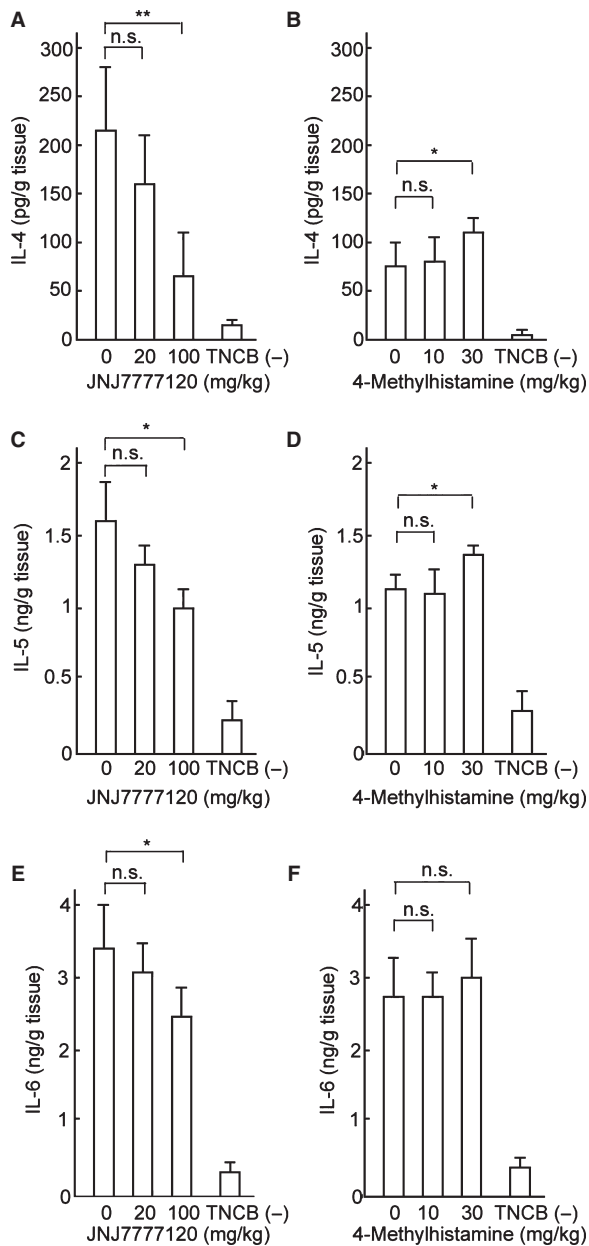


Figure 4. Effect of H₄ receptor activation on Th2 cytokines in eczematous lesions induced by repeated challenge. The procedure for the application of 2,4,6-trinitro-1-chlorobenzene, JNJ7777120, and 4-methylhistamine was the same as described in Fig. 2A. Interleukin (IL)-4 (A, B), IL-5 (C, D), IL-6 (E, F) of histidine decarboxylase (HDC) (+/+) mice (A, C, E) and HDC (-/-) mice (B, D, F). Data are expressed as mean $\pm$ SD ($n = 6$). n.s., not significant. * $P < 0.05$, ** $P < 0.01$.

Productions of Th1 cytokines are decreased by H₄ receptor activation

IFN- γ levels in eczematous lesions of HDC (+/+) mice were slightly increased by treatment with JNJ7777120, while levels in HDC (-/-) mice were slightly decreased by

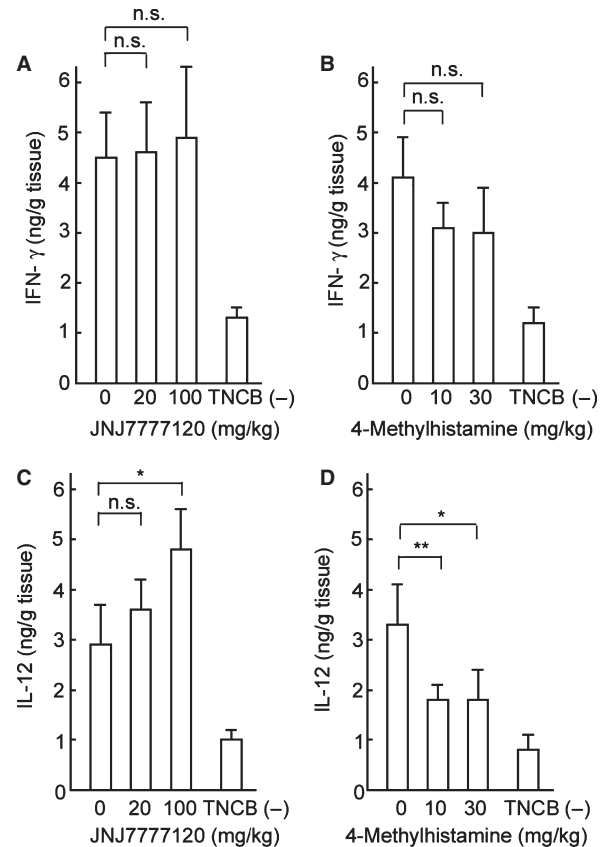


Figure 5. Effect of H₄ receptor activation on Th1 cytokines. Interferon- γ of histidine decarboxylase (HDC) (+/+) mice (A) and HDC (-/-) mice (B). Interleukin-12 of HDC (+/+) mice (C) and HDC (-/-) mice (D). Data are expressed as mean $\pm$ SD ($n = 6$). n.s., not significant. * $P < 0.05$, ** $P < 0.01$.

treatment with 4-methylhistamine (Fig. 5A,B). IL-12 levels in eczematous lesions of HDC (+/+) mice were significantly increased by treatment with 100 mg/kg of JNJ7777120, while levels in HDC (-/-) mice were significantly decreased by treatment with 1 μ mol of 4-methylhistamine (Fig. 5C,D). Therefore, unlike Th-2 cytokines, Th-1 cytokines tend to be decreased by H₄ receptor signaling.

Production of serum immunoglobulin (Ig)E is increased by H₄ receptor activation

Although serum IgE levels in both HDC (+/+) and HDC (-/-) mice were increased by repeated challenge, IgE levels in HDC (+/+) mice were always higher than those in HDC (-/-) mice (Fig. 6A,B). Serum IgE levels in HDC (+/+) mice were significantly decreased by treatment with 100 mg/kg of JNJ7777120, while levels in HDC (-/-) mice were significantly increased by treatment with 1 μ mol of 4-methylhistamine (Fig. 6A,B).

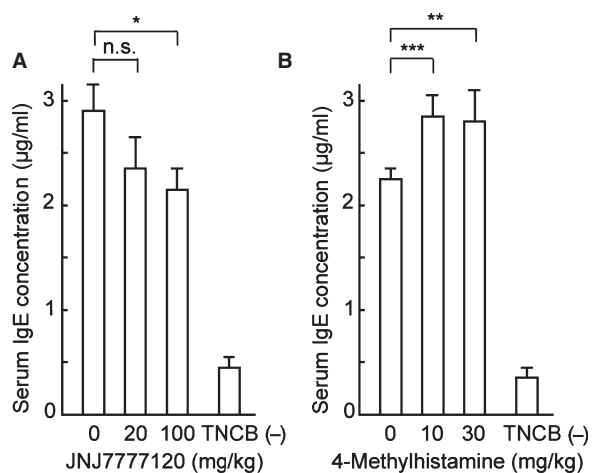


Figure 6. Effect of H_4 receptor activation on serum IgE concentration. Histidine decarboxylase (HDC) (+/+) mice. (B) HDC (-/-) mice. JNJ7777120 and 4-methylhistamine were administered in HDC (+/+) and HDC (-/-) mice, respectively, everyday during repeated challenge 30 min before the challenge. JNJ7777120 or 4-methylhistamine was administered ten times as total. Data are expressed as mean $\pm$ SD ($n = 6$). n.s., not significant. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

Discussion

Epicutaneous application of contact sensitizing agents is able to induce contact hypersensitivity response, as a delayed-type hypersensitivity, when challenge with the agent is performed a second time (15). H_1 receptor antagonist inhibits murine contact hypersensitivity response (16), but with insufficient efficacy. H_2 receptor antagonist augments the contact hypersensitivity response (17). Histamine H_4 receptor antagonism reduces hapten-induced scratching behavior but not ear swelling (18). Repeated challenge, inducing immediate-type hypersensitivity and a shift in the cutaneous cytokine milieu from T-helper cell type (Th)1 to Th2 profiles (10), develops chronic allergic contact dermatitis. However, the influence of H_4 receptor has not been investigated using repeated challenge. We therefore further investigated the effects of H_4 receptor antagonist in repeated challenge using two types of hapten challenge procedure, which have been proclaimed to represent delayed-type and chronic allergic contact dermatitis (9, 10).

Swelling measured 24 h after the challenge in the contact hypersensitivity skin reaction (Fig. 1A) was not influenced by JNJ7777120 in HDC (+/+) mice, as Rossbach et al. reported (18), or by 4-methylhistamine in HDC (-/-) mice. Small numbers of mast cells and eosinophils were observed in the dermis after single challenge, but numbers were not markedly influenced by administration of H_4 antagonist. The H_4 receptor thus does not seem to be strongly involved in delayed-type hypersensitivity responses. The overall role of histamine

in delayed-type hypersensitivity responses is not prominent, as we have previously reported (19).

In allergic skin diseases, most inflammation is caused by chronic introduction of antigens into the skin. In patients with chronic contact dermatitis, repeated exposure to antigens through the skin is thought to contribute to the development of eczematous lesions. Repeated application of TNCB results in antigen-specific hypersensitivity responses from a delayed- to an early-type response, accumulation of mast cells in the upper part of the dermis and elevation of serum IgE levels (9). Atopic dermatitis, aggravated by chronic exposure to antigens, is a common and distinctive form of allergic skin diseases associated with eczematous lesions, early-type hypersensitivity responses, and increased IgE production in response to environmental allergens (20, 21). Atopic dermatitis bears clinical, histological, and immunological similarities to chronic allergic contact dermatitis (22, 23). In our studies, H_4 receptor antagonist decreased the numbers of mast cells and eosinophils specifically in regions of repeated epicutaneous challenge in HDC (+/+) mice, while H_4 receptor agonist increased counts for these cells. Histamine thus seems to act as a chemotactic agent for mast cells and eosinophils in eczematous lesions via the H_4 receptor. H_4 receptor was newly expressed in eczematous lesions. This finding might reflect the observed mast cell and eosinophil infiltration, because these cells express H_4 receptors on the cell membrane (3, 6). H_4 receptor mediates histamine-induced chemotaxis of mast cells in mouse *in vitro* (5, 6). Histamine-induced migration of mast cells is also observed *in vivo*, with histamine inhalation leading to increases in total and sub-epithelial numbers of mast cells in the murine trachea (6). In TNCB-treated skin, we could observe the expression of H_4 receptor, which was not immunohistochemically detected in control skin. The expression of H_4 receptor would be up-regulated by repeated application of TNCB. H_4 receptor was considered to express on mast cells, as it was expressed on IgE-positive cells in eczematous lesions. Although eosinophils are reported to express H_4 receptor and be infiltrated by histamine through the H_4 receptor (3), we did not immunohistochemically detect the expression of H_4 receptor on eosinophils. Thioperamide, a histamine H_4/H_3 receptor antagonist, inhibits mast cell and eosinophil migration into guinea pig esophageal epithelium induced by antigen inhalation (24). Given the background information, we considered that repeated exposure to antigen-induced mast cell and eosinophil infiltration through H_4 receptor.

Scratching behavior was exclusively observed to the ears or the back challenged with allergens in HDC (+/+) mice, whereas it was barely observed in HDC (-/-) mice (25). In the present study, scratching was attenuated by treatment with JNJ7777120, as Dunford et al. are reported (26). As scratching would contribute to the

development of the eczematous lesions, it might be one of the most important factors to induce mast cell and eosinophil infiltration.

Evidence has been emerging that innate immune lymphocytes such as invariant Natural Killer T (NKT) (iNKT) cells and even Natural Killer (NK) cells may play important roles in contact dermatitis (27, 28). Because we reported that activation of H₄ receptor on iNKT cells would transmit the information to produce IL-4 (29), control of IL-4 production *in vivo* might reside with iNKT cells. Dendritic cells play a predominant role in allergic immune response and prime naïve T cells into Th2 type with high IL-4-producing capacity (30). Because blockade of the H₄ receptor on dendritic cells limits their ability to induce Th2 responses (31), the control of IL-4 production in our experiment might be attributed to Th2 cells as well. IL-4 may also come from mast cells or eosinophils during skin reaction. The elevated IL-5 might augment eosinophil infiltration in the lesion in addition to the direct histamine chemotactic activity through the H₄ receptor, because IL-5 is chemotactic for eosinophils and activates mature eosinophils. IL-4 and IL-5 are produced in mast cells and Th2 cells, whereas IL-6 is produced in monocytes and macrophages. Such differences in sources might be the reason why H₄ receptor activation induced IL-4 and IL-5 production more strongly than IL-6 production in our studies, as infiltration of mast cells, but not of monocytes or macrophages was dominant in the lesion.

H₄ receptor did not significantly influence levels of IFN- γ . Although H₄ receptor agonist decreased levels of IL-12 in eczematous lesions, H₄ receptor antagonist increased these levels. Because IL-12 is an important factor in inducing differentiation of Th1 response, sup-

pressive effects of histamine on IL-12 level through the H₄ receptor might contribute to shifts from a Th1 to a Th2 response. The Th1 to Th2 shift observed in repeated TNCB application (10) could thus be transmitted through H₄ receptors. In our result, serum IgE levels were decreased by treatment with H₄ receptor antagonist. IL-4 is known to play the central role in IgE induction (32), so signaling effect through H₄ receptor on IL-4 production might be one of the mechanisms explaining high IgE production in our result. In human, severe or obvious atopy correlates with the high levels of serum IgE and an increased number of eosinophils and mast cells in lesions (33). As atopic dermatitis lesions become more chronic, they exhibit increased expression of IFN- γ , IL-12, and GM-CSF (34). The mechanism behind this switch from Th2 to Th1 is not well understood. Even though we repeated TNCB challenge to produce this model, the cytokine expression profile were Th2 type, therefore the atopic dermatitis in this experiment might be classified to 'acute lesions' in human atopic dermatitis. Taken together, H₄ receptor antagonist might be useful for treating these diseases, which is deteriorated or induced by repeated exposure to antigens. However, further studies to clarify H₄ function in chronic allergic contact dermatitis and atopic dermatitis are needed before the antagonist could be used clinically.

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References

- Oda T, Morikawa N, Saito Y, Maruho Y, Yamamoto S. Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J Biol Chem* 2000; **275**:36781–36786.
- Liu C, Wilson SJ, Kuei C, Lovenberg TW. Comparison of human, mouse, rat and guinea pig histamine H₄ receptors reveals substantial pharmacological species variation. *J Pharmacol Exp Ther* 2001; **299**:121–130.
- Ling P, Ngo K, Nguyen S, Thurmond RL, Edwards JP, Karlsson L et al. Histamine H₄ receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. *Br J Pharmacol* 2004; **142**:161–171.
- Buckland KF, Williams TJ, Conroy DM. Histamine induces cytoskeletal changes in human eosinophils via the H(4) receptor. *Br J Pharmacol* 2003; **140**:1117–1127.
- Hofstra CL, Desai PJ, Thurmond RL, Fung-Leung WP. Histamine H₄ receptor mediates chemotaxis and calcium mobilization of mast cells. *J Pharmacol Exp Ther* 2003; **305**:1212–1221.
- Thurmond RL, Desai PJ, Dunford PJ, Fung-Leung WP, Hofstra CL, Jiang W et al. A potent and selective histamine H₄ receptor antagonist with anti-inflammatory properties. *J Pharmacol Exp Ther* 2004; **309**:404–413.
- Dijkstra D, Leurs R, Chazot P, Shenton FC, Stark H, Werfel T et al. Histamine downregulates monocyte CCL2 production through the histamine H₄ receptor. *J Allergy Clin Immunol* 2007; **120**:300–307.
- Dijkstra D, Stark H, Chazot PL, Shenton FC, Leurs R, Werfel T et al. Human inflammatory dendritic epidermal cells express a functional histamine H(4) receptor. *J Invest Dermatol* 2008; **128**:1696–1703.
- Kitagaki H, Fujisawa S, Watanabe K, Hayakawa K, Shiohara T. Immediate-type hypersensitivity response followed by a late reaction is induced by repeated epicutaneous application of contact sensitization agents in mice. *J Invest Dermatol* 1995; **105**:749–755.

10. Kitagaki H, Ono N, Hayakawa K, Kitazawa T, Watanabe K, Shiohara T. Repeated elicitation of contact hypersensitivity induces a shift in cutaneous cytokine milieu from a T helper cell type 1 to a T helper cell type 2 profile. *J Immunol* 1997;**159**:2484–2491.
11. Seike M, Takata T, Ikeda M, Kodama H, Terui T, Ohtsu H. Histamine helps development of eczematous lesions in experimental contact dermatitis in mice. *Arch Dermatol Res* 2005;**297**:68–74.
12. Hamada R, Seike M, Kamijima R, Ikeda M, Kodama H, Ohtsu H. Neuronal conditions of spinal cord in dermatitis are improved by olopatadine. *Eur J Pharmacol* 2006;**547**:45–51.
13. Ohtsu H, Tanaka S, Terui T, Hori Y, Makabe-Kobayashi Y, Pejler G et al. Mice lacking histidine decarboxylase exhibit abnormal mast cells. *FEBS Lett* 2001;**502**:53–56.
14. Oka S, Wakui J, Ikeda S, Yanagimoto S, Kishimoto S, Gokoh M et al. Involvement of the cannabinoid CB2 receptor and its endogenous ligand 2-arachidonoylglycerol in oxazolone-induced contact dermatitis in mice. *J Immunol* 2006;**177**:8796–8805.
15. Gray D. Immunological memory. *Annu Rev Immunol* 1993;**11**:49–77.
16. Tokura Y, Kobayashi M, Ito T, Takahashi H, Matsubara A, Takigawa M. Anti-allergic drug olopatadine suppresses murine contact hypersensitivity and downmodulates antigen-presenting ability of epidermal Langerhans cells. *Cell Immunol* 2003;**224**:47–54.
17. Belsito DV, Kerdel FA, Potozkin J, Soter NA. Cimetidine-induced augmentation of allergic contact hypersensitivity reactions in mice. *J Invest Dermatol* 1990;**94**:441–445.
18. Rossbach K, Wendorff S, Sander K, Stark H, Gutzmer R, Werfel T et al. Histamine H4 receptor antagonism reduces hapten-induced scratching behaviour but not inflammation. *Exp Dermatol* 2009;**18**:57–63.
19. Ohtsu H, Kuramasu A, Tanaka S, Terui T, Hirasawa N, Hara M et al. Plasma extravasation induced by dietary supplemented histamine in histamine-free mice. *Eur J Immunol* 2002;**32**:1698–1708.
20. Cooper KD. Atopic dermatitis: recent trends in pathogenesis and therapy. *J Invest Dermatol* 1994;**102**:128–137.
21. Ohmen JD, Hanifin JM, Nickoloff BJ, Rea TH, Wyzykowski R, Kim J et al. Overexpression of IL-10 in atopic dermatitis. Contrasting cytokine patterns with delayed-type hypersensitivity reactions. *J Immunol* 1995;**154**:1956–1963.
22. Wang G, Savinko T, Wolff H, Dieu-Nosjean MC, Kemeny L, Homey B et al. Repeated epicutaneous exposures to ovalbumin progressively induce atopic dermatitis-like lesions in mice. *Clin Exp Allergy* 2007;**37**:151–161.
23. Man MQ, Hatano Y, Lee SH, Man M, Chang S, Feingold KR et al. Characterization of a hapten-induced, murine model with multiple features of atopic dermatitis: structural, immunologic, and biochemical changes following single versus multiple oxazolone challenges. *J Invest Dermatol* 2008;**128**:79–86.
24. Yu S, Stahl E, Li Q, Quyang A. Antigen inhalation induces mast cells and eosinophils in the guinea pig esophageal epithelium involving histamine-mediated pathway. *Life Sci* 2008;**82**:324–330.
25. Seike M, Ikeda M, Kodama H, Terui T, Ohtsu H. Inhibition of scratching behaviour caused by contact dermatitis in histidine decarboxylase gene knock-out mice. *Exp Dermatol* 2005;**14**:169–175.
26. Dunford PJ, Williams KN, Desai PJ, Karisson L, McQueen D, Thurmond RL. Histamine H₄ receptor antagonists are superior to traditional antihistamines in the attenuation of experimental pruritus. *J Allergy Clin Immunol* 2007;**119**:176–183.
27. Campos RA, Szczepanik M, Itakura A, Akahira-Azuma M, Sidobre S, Kronenberg M et al. Cutaneous immunization rapidly activates liver invariant α 14 NKT cells stimulating B-1 B cells to initiate T cell recruitment for elicitation of contact sensitivity. *J Exp Med* 2003;**198**:1785–1796.
28. O'Leary JG, Goodarizi M, Drayton DL, von Andrian UH. T cell- and B cell-independent adaptive immunity mediated by natural killer cells. *Nat Immunol* 2006;**7**:507–516.
29. Leite-de-Moraes MC, Diem S, Michel ML, Ohtsu H, Thurmond RL, Schneider E et al. Cutting edge: Histamine receptor H4 activation positively regulates in vivo IL-4 and IFN- γ production by invariant NKT cells. *J Immunol* 2009;**182**:1233–1236.
30. Novak N, Valenta R, Bohle B, Laffer S, Haberkost J, Kraft S et al. Fc ϵ RI engagement of Langerhans cell-like dendritic cells and inflammatory dendritic epidermal cell-like dendritic cells induces chemotactic signals and different T-cell phenotypes in vitro. *J Allergy Clin Immunol* 2004;**113**:949–957.
31. Dunford PJ, O'Donnell N, Riley JP, Williams KN, Karlsson L, Thurmond RL. The histamine H4 receptor mediates allergic airway inflammation by regulating the activation of CD4⁺ T cells. *J Immunol* 2006;**176**:7062–7070.
32. Kühn R, Rajewsky K, Müller W. Generation and analysis of interleukin-4 deficient mice. *Science* 1991;**254**:707–710.
33. Kajosaari M, Saarinen UM. Evaluation of laboratory tests in childhood allergy. Total serum IgE, blood eosinophilia and eosinophil and mast cells in nasal mucosa of 178 children aged 3 years. *Allergy* 1981;**36**:329–335.
34. Hamid Q, Boguniewicz M, Leung DY. Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. *J Clin Invest* 1994;**94**:870–876.