



The antihistamine olopatadine regulates T cell activation in palladium allergy

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ABSTRACT

Because of its corrosion resistance palladium (Pd) has been widely used in many consumer products ranging from fashion accessories to dental materials. Recently, however, an increase in Pd allergy cases has been reported. Metal allergy is categorized as a Type IV allergy, which is characterized as a delayed-type hypersensitivity reaction in which T cells are known to play an important role; however, the precise mechanism of their action remains unclear. Here we defined the relationship between histamine and the Pd allergic reaction specifically with respect to T cell responses. To verify the effects of histamine on T cells, we examined whether there is a change in IFN- γ production following stimulation of histamine or the antihistamine, olopatadine hydrochloride (OLP), *in vitro*. In addition, we assessed whether OLP administration affected the degree of footpad swelling or IFN- γ production during the Pd allergy response in mice. We found that histamine stimulation increased IFN- γ production in T cells, specifically enhancing IFN- γ production in CD8⁺ T cells compared with CD4⁺ T cells. Interestingly, OLP suppressed the production of IFN- γ in CD8⁺ T cells, and this compound inhibited footpad swelling and IFN- γ production in mice with Pd allergy. These results suggest that histamine promotes the Type IV allergic reaction and thus, the histamine 1 receptor (H1R) might be useful therapeutic target for treatment of metal allergy.

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1. Introduction

The main symptoms of metal allergy are redness, swelling, and itching. Common jewelry including earrings, necklaces, and watches are a major cause of metal allergy, while metallic material used in medical treatments such as dental restorations and surgical instruments may also cause reactions. Recently, the number of patients with metal allergy has increased due to wide use of metal in production of jewelry, surgical instruments, and dental restorations [1]. While nickel, cobalt, and chromium are well known to be causal agents of metal allergy, reactions to palladium (Pd) are also becoming a serious problem [2,3].

Metal allergy manifests as a delayed-type hypersensitivity reaction and is categorized as a type IV allergy. Although the onset mechanism is thought to be the uptake of metal ions into the body followed by antigen presentation leading to excessive cellular immune reactions, the precise underlying mechanisms remain unclear [4–6]. One of the reasons for this lack of understanding is the absence of established animal

models for Pd allergy. Although two mouse models of Pd allergy are currently known [7,8], it takes a long time to master the technical skills for preparation of these models. Therefore, we designed an improved mouse model of Pd allergy by altering this protocol to include two sensitizations and a single footpad challenge.

While T cells are known to be important in the delayed-type hypersensitivity reaction, their precise role is unclear [9–11]. Recently, Histamine has been reported to regulate inflammation [12], allergic dermatitis [13], and the function of T cells during immune responses [14]. Histamine is released from mast cells, basophils, and many other cell types and functions to increase vascular permeability, vasodilation, and release of inflammatory cytokines that result in the promotion of inflammatory reactions such as swelling, redness, and itching of the lesion. Among the four histamine receptor types (H1, H2, H3, and H4), the histamine H1 receptor (H1R) is known to be involved in allergic reactions and itching, and is expressed by many cells including vascular endothelial cells, mast cells, neutrophils, basophils, dendritic cells, B cells, and T cells [15,16]. Currently, antihistamines are prescribed as an adjunct therapy for itching in metal allergy patients. One commonly prescribed second-generation antihistamine is olopatadine hydrochloride (OLP), which is known to selectively block the H1R, resulting in suppression of inflammatory cytokine production, such as IL-6 and TNF- α [17,18].

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In this study, we improved the Pd allergy mouse model and examined the effects of histamine and OLP on the immune response during Pd allergy, with particular focus on T cell function.

2. Materials and methods

2.1. Ethics statement

Mice were maintained under specific pathogen-free conditions, and all procedures were performed according to the protocols approved by the Institutional Committee for Use and Care of Laboratory Animals of Tohoku University, which was granted by Tohoku University Ethics Review Board (No. 2012AcA-069), and the Guide for Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication 85-23, revised 1996). All surgeries were performed under anesthesia via isoflurane (Merck & Co., Inc., Whitehouse Station, N.J., USA). For collection of tissue samples mice were sacrificed by cervical dislocation. All efforts were made to minimize suffering.

2.2. Mice

BALB/c mice (7-week-old females) were obtained from CLEA Japan (Tokyo, Japan) and were maintained under specific pathogen-free conditions. Animal experiments were approved by the Institutional Committee for Use and Care of Laboratory Animals of Tohoku University.

2.3. Reagents

PdCl₂ (purity: 99.9%) purchased from Wako Pure Chemical Industries (Osaka, Japan) was dissolved in sterile saline. Lipopolysaccharide (LPS) prepared from *Escherichia coli* O55:B5 by phenol water extraction was purchased from Sigma (St Louis, MO, USA) and dissolved in phosphate buffered saline (PBS). Olopatadine hydrochloride (OLP; 11-[(Z)-3-(dimethylamino)-propylidene]-6,11-dihydrodibenz[b,e]-oxepin-2-acetic acid monohydrochloride) (purity >95%) was purchased from Tokyo Kasei (Tokyo, Japan).

2.4. Induction of Pd allergy and measurement of allergic footpad swelling

For sensitization, 125 µL of 10 mM PdCl₂ with 10 µg/mL LPS in PBS was injected twice into the left and right groin of mice at an interval of 7 days via the intradermal (i.d.) route (250 µL per mouse) [19]. At day 7 after the 2nd sensitization, mice were challenged by i.d. injection of 25 µL of 10 mM PdCl₂ (without LPS) in PBS into both the left and right footpads. For mock sensitization as a control, mice were injected with PBS and then challenged with 10 mM PdCl₂ (without LPS). Footpad swelling was measured before and at 3–72 h after the challenge using a Peacock Dial Thickness Gauge (Ozaki MFG Co. Ltd, Tokyo, Japan) and the difference in footpad thickness before and after challenge was calculated.

2.5. Histological analysis

Pd allergy mouse model and control mice were sacrificed at 24 h after the challenge. Footpad samples fixed in 10% neutral buffered formalin solution, decalcified with 10% formic acid, and embedded in paraffin. Sections were cut with a microtome, deparaffinized, rehydrated in a graded series of ethanol, and stained with hematoxylin and eosin (H&E).

2.6. Olopatadine hydrochloride (OLP) administration

Pd allergy was induced in mice as described above. Mice were injected intraperitoneally (i.p.) with OLP once per day on days –1 to 14. On the day of sensitization (days 0 and 7) and challenge (day 14), OLP was injected 1 h prior to the sensitizations or challenge. The dose

of OLP was 1 mg/kg of weight in 200 µL of PBS. As a control, mice were injected i.p. with PBS instead of OLP [20].

2.7. Flow cytometric analysis of cytokine production

Mice were sacrificed before and at 3–72 h after the challenge, and then lymphocytes were isolated from splenocytes and popliteal lymph nodes. These cells were stimulated with 20 ng/mL phorbol myristate acetate (PMA) (Sigma Aldrich) plus 0.5 µg/mL ionomycin (Sigma Aldrich) and were then incubated with GolgiStop (BD Biosciences) for 5 h at 37 °C in a humidified atmosphere of 5% CO₂. After washing twice with 3% FCS-supplemented RPMI-1640, the cells were pretreated with anti-CD16 + CD32 mAb (2.4G2) (BD Biosciences) to block Fc receptors and were then stained with a combination of fluorescein isothiocyanate (FITC)-labeled anti-CD8 (53–6.7) and allophycocyanin (APC)-labeled anti-CD4 (GK1.5). These antibodies were purchased from Bio Legend (San Diego, CA, USA). The cells were then fixed and permeabilized with BD Cytofix/Cytoperm solution (BD Biosciences) according to manufacturer's instructions followed by staining with PE-labeled anti-IFN-γ (XMG1.2), anti-IL-4 (11B11), anti-IL-10 (JES5-16E3), or anti-IL-17 (TC11-18H10.1) mAbs. The cells were washed twice with BD Perm/Wash buffer (BD Biosciences) and then analyzed on a FACSCanto II (BD Biosciences).

2.8. T cell stimulation with histamine in vivo

Mice were made midline abdominal laparotomy, and injected directly 100 µL of 30 mmol/L histamine into the spleen, followed by abdominal closure. As a control, mice were injected with PBS. Mice were sacrificed at 12 h after the injection, and then lymphocytes were isolated from splenocytes. Production of IFN-γ in T cells was analyzed on a FACSCanto II (BD Biosciences).

2.9. T cell stimulation with histamine and OLP in vitro

Cells were isolated from splenocytes and B cells were removed using BioMag Goat Anti-Mouse IgG (QIAGEN Tokyo, Japan). T cells were then cultured with rIL-2 (30 U/mL; Wako Pure Chemical Industries, Ltd. Osaka, Japan) and Mouse T-Activator anti-CD3 and anti-CD28 antibody-coated beads (Life Technologies, USA) for 3 days according to the manufacturer's instructions. In the histamine stimulation group, histamine (30 µmol/L) was added for 24 h (from day 2 to 3 of culture). In the OLP stimulation group, histamine (30 µmol/L) was added for 24 h and OLP (300 nmol/L) was added for 4 h on day 3 of culture. After washing twice with 3% FCS-supplemented RPMI-1640, the cells were stimulated with PMA and ionomycin with GolgiStop for 5 h at 37 °C in a humidified atmosphere of 5% CO₂. Cells were analyzed on a FACSCanto II (BD Biosciences).

2.10. Quantitative PCR of histamine 1 receptor (H1R)

The mRNA of Histamine 1 receptor (H1R) in T cells was analyzed using quantitative RT-PCR. Cells were isolated from popliteal lymph nodes and splenocytes, and then B cells were removed. Furthermore, T cells were separated into CD4⁺ and CD8⁺ using magnetic activated cell sorting (MACS) beads (Miltenly Biotec, Bergisch Glad bech, Germany). T cells were cultured with 10% FCS-supplemented RPMI-1640 with or without histamine (30 µmol/L) for 24 h. Total RNA from T cells was extracted using the RNeasy lipid tissue kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Total RNA was reverse transcribed using SuperScript III with Oligo (dT)_{12–18} as the primer (Invitrogen, Carlsbad, CA, USA) and the reverse transcribed sample was diluted 1:10 and used as a cDNA template. Quantitative RT-PCR was carried out in a DNA chromo 4 (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. Specific PCR primers were as follows: Histamine 1 receptor (H1R): sense 5'-GGG CTC AAA

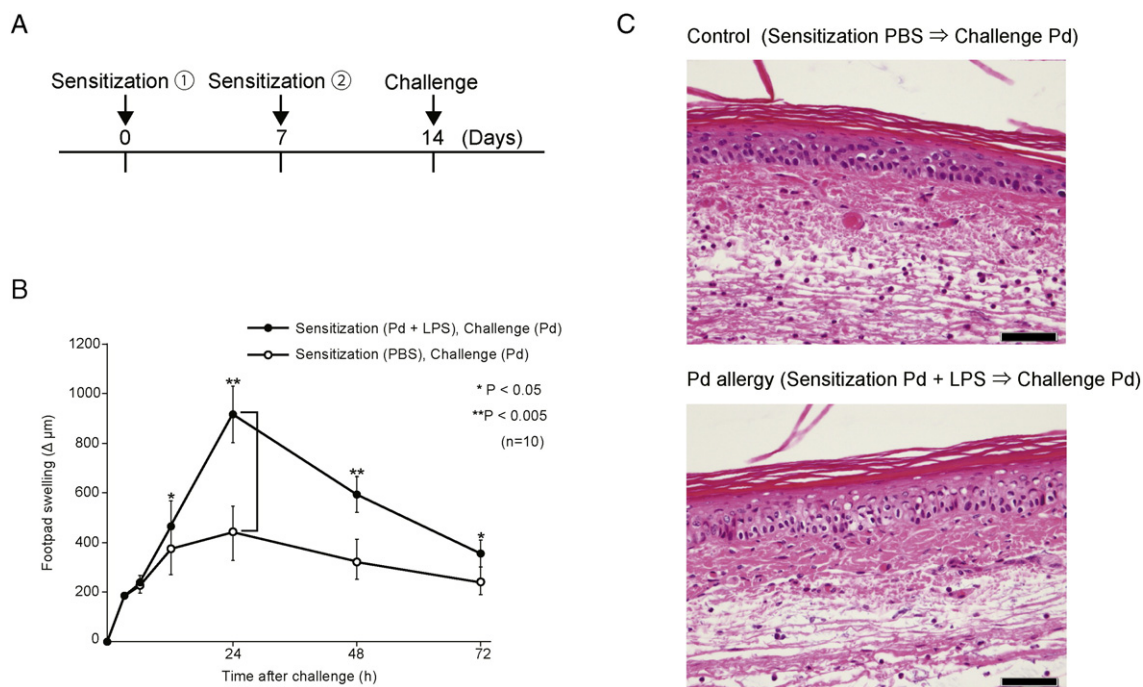


Fig. 1. Induction of Pd allergy and measurement of allergic footpad swelling, and histological analysis. (A) Induced Pd mouse allergy model. BALB/c mice (7-week-old females) were sensitized twice with PdCl₂ and LPS into the left and right groin at an interval of 7 days via the intradermal (i.d.) route. At day 7 after the 2nd sensitization, mice were challenged by i.d. injection of PdCl₂ (without LPS) in PBS into the left and right footpads. As a control, mice were injected with PBS for mock sensitization and PdCl₂ (without LPS) as the challenge. (B) Footpad swelling was measured before and at 3–72 h after the challenge. The difference in footpad thickness before and after challenge was calculated. Closed circles (●) represent Pd allergy mice, while open circles (○) represent the control. In both groups the peak in footpad swelling was observed at 24 h after the challenge with Pd. Data are represented as the mean $\pm$ SD (n = 10). Statistical significance was tested by unpaired Student's *t*-test (* $p < 0.05$, ** $p < 0.005$). (C) Mice were sacrificed at 24 h after the challenge. Mice were injected with PBS for mock sensitization as a control, and then challenged with PdCl₂. The tissue sections were stained with hematoxylin and eosin (H&E; Scale bars, 50 μm).

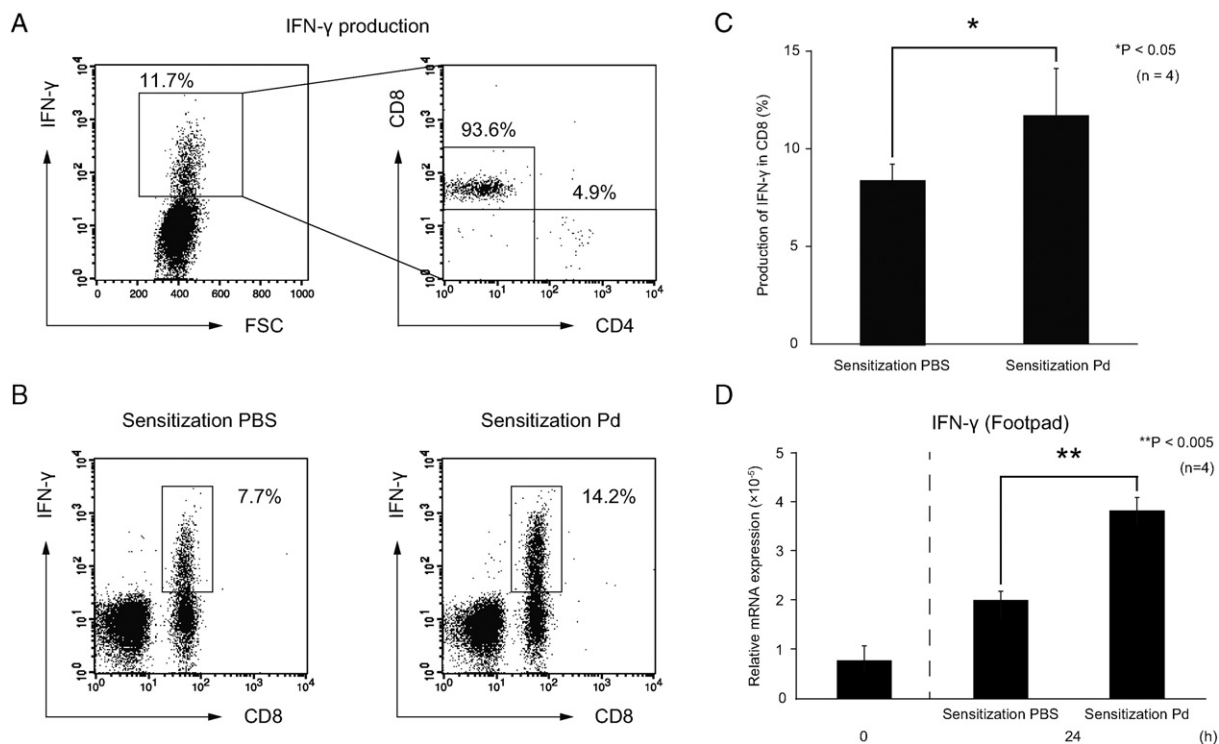


Fig. 2. IFN- γ production in Pd allergy mice. Mice were sacrificed at 15 h after the challenge, and then lymphocytes were isolated from popliteal lymph nodes and IFN- γ production was analyzed using Flow cytometry. (A) The left panel shows IFN- γ production within total lymphocytes. The right panel shows the percentage of IFN- γ producing CD4⁺ or CD8⁺ T cells. (B) IFN- γ production in CD8⁺ T cells from the PBS-sensitized and the Pd-sensitized groups. (C) Statistical analysis of IFN- γ production in CD8⁺ T cells. Percentages were calculated by setting total lymphocytes as 100%. (D) Mice were sacrificed before and at 24 h after the challenge. IFN- γ in footpad was assessed by quantitative RT-PCR. (C and D) Error bars indicate mean $\pm$ SD (n = 4). Statistical significance was tested by unpaired Student's *t*-test (* $p < 0.05$, ** $p < 0.005$).

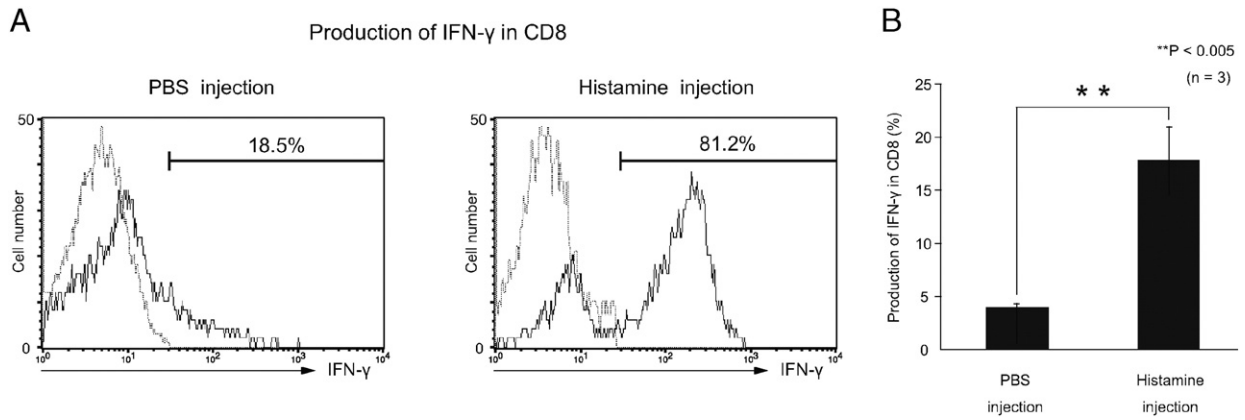


Fig. 3. In vivo stimulation of T cells with histamine. Histamine was injected directly into the spleen of mice, and the mice were sacrificed at 12 h after the injection, and then lymphocytes were isolated from splenocytes. As a control, mice were injected with PBS. (A) Production of IFN-γ in CD8⁺ T cells was analyzed using Flow cytometry. CD8⁺ T cells were stained with isotype control mAb (dotted line histograms) or anti-IFN-γ mAb (solid line histograms). The percentage shows IFN-γ producing CD8⁺ T cells. (B) Statistical analysis of IFN-γ production in CD8⁺ T cells. Percentages were calculated by setting total lymphocytes as 100%. Error bars indicate mean ± SD (n = 3). Statistical significance was tested by unpaired Student's *t*-test (***p* < 0.005).

GGC CAA TGA C-3', antisense 5'-TCC GCC GGC AAG TAC TCA-3'; GAPDH: sense 5'-TGA AGG TCG GTG TGA ACG GAT TTG GC-3', antisense 5'-CAT GTA GGC CAT GAG GTC CAC CAC-3'. SYBR® green was used for quantification of the amplified DNA. Cycling conditions were: 94 °C for 3 min, followed by 40 cycles of 94 °C for 15 s, 57 °C for 25 s, and 72 °C for 15 s. At the end of the run, the melting step was performed with slow heating from 55 °C to 95 °C at a rate of 0.5 °C per 10 s with continuous measurement of fluorescence. Data were analyzed using Opticon Monitor version 3 software (Bio-Rad laboratories).

2.11. Quantitative PCR of IFN-γ

Mice were sacrificed before and at 24 h after the challenge. Total RNA from footpad was isolated and expressions of IFN-γ were assessed

by quantitative RT-PCR. Specific PCR primers were as follows: IFN-γ: sense 5'-CGG CAC AGT CAT TGA AAG CCT A-3', antisense 5'-GTT GCT GAT CTG ATT GTC-3'. SYBR® green was used for quantification of the amplified DNA. Cycling conditions were: 95 °C for 30 s, followed by 44 cycles of 95 °C for 1 s, 60 °C for 5 s, and 72 °C for 2 s. According to the manufacturer's instructions, data were analyzed using Opticon Monitor version 3 software (Bio-Rad laboratories).

2.12. Statistical analysis

Statistical significance with respect to change in footpad swelling and IFN-γ production for each group was analyzed using Student's *t*-test. **p* ≤ 0.05 and ***p* ≤ 0.005 were considered to be significant.

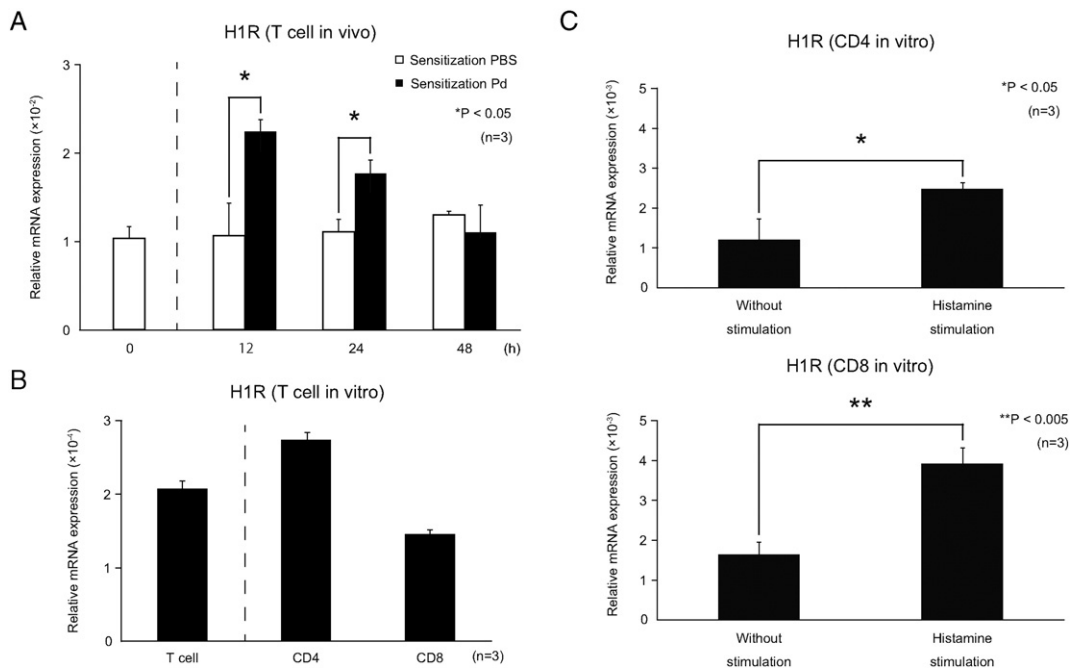


Fig. 4. Expression of histamine 1 receptor (H1R) in T cells. (A) H1R is expressed in T cells *in vivo*. Mice were sacrificed before and at 12, 24, and 48 h after the challenge, and the cells were isolated from popliteal lymph nodes and B cells were removed. (B and C) H1R is expressed in T cells *in vitro*. Cells were isolated from splenocytes and B cells were removed. In addition, T cells were separated into CD4⁺ and CD8⁺. (C) T cells were cultured in 10% FCS-supplemented RPMI-1640 with or without histamine for 24 h. Total RNA was isolated and expressions of H1R were assessed by quantitative RT-PCR. Error bars indicate mean ± SD (n = 3). Statistical significance was tested by unpaired Student's *t*-test (**p* < 0.05, ***p* < 0.005).

3. Results

3.1. Footpad swelling in Pd allergy mice

To demonstrate the induction of Pd allergy in a mouse model, we evaluated the inflammatory response *via* footpad swelling. Mice were sensitized twice with PdCl₂ and LPS, and then challenged once with PdCl₂ (Fig. 1A) and footpad swelling was measured before and at 3–72 h after the challenge. In both groups, the peak of footpad swelling was observed at 24 h after challenge (Fig. 1B); however, footpad swelling was significantly enhanced in the Pd-sensitized group compared with the PBS-sensitized group at 24 h after the challenge. The difference in footpad swelling existed between the Pd-sensitized and PBS-sensitized groups even at 12, 48, and 72 h after Pd challenge. Furthermore, Pd-sensitized mice were found to be slow in recovery from footpad swelling when compared with PBS-sensitized mice at 72 h.

In histological analysis, both Pd allergy and control groups showed the increase of lymphocytes infiltration into epidermis. However, vacuolar degeneration was significantly increased in the Pd-sensitized group compared with the PBS-sensitized group (Fig. 1C).

Thus, sensitization with Pd prior to challenge produced an inflammatory response similarly to that seen during the allergic response to this metal in human, suggesting that this is an appropriate model system.

3.2. Cytokine production in Pd allergy mice

To clarify which cytokines contribute to the development of Pd allergy, we analyzed cytokine production (IL-4, IL-10, IL-17, and IFN- γ) in cells using Flow cytometry. After mice were sacrificed before and at 3–72 h after challenge, lymphocytes were isolated from splenocytes and popliteal lymph node cells. No production of IL-4, IL-10, and IL-17 was detected in splenocytes or popliteal lymph node cells (data not shown). In contrast, IFN- γ was produced from splenocytes in Pd-sensitized mice as well as PBS-sensitized mice (data not shown). To determine which T cell subset contributes to IFN- γ production, we assessed CD4⁺ and CD8⁺ T cells in popliteal lymph nodes at 15 h after Pd challenge and found that 93.6% of IFN- γ producing cells were CD8⁺ T cells (Fig. 2A). Furthermore, IFN- γ production in CD8⁺ T cells was significantly increased in the Pd-sensitized group compared with the PBS-sensitized group (Fig. 2B and C).

Then, we analyzed the IFN- γ production from the footpad using quantitative RT-PCR. The amount of IFN- γ in the Pd-sensitized group was significantly increased compared with the PBS-sensitized group (Fig. 2D).

Additionally, to clarify histamine contribution to IFN- γ production in CD8⁺ T cells, we analyzed cells using Flow cytometry. The production of IFN- γ in CD8⁺ T cells was significantly increased in the histamine-injected group compared with the PBS-injected group (Fig. 3). These results suggest that IFN- γ produced by CD8⁺ T cells contributes to the development of Pd allergy and that histamine is important for the induction of IFN- γ production in CD8⁺ T cells.

3.3. Expression of histamine 1 receptor (H1R) in T cells

Recently, histamine was reported to regulate the function of T cells during immune responses. Thus, to verify the effects of histamine on T cells, we examined H1R expression in T cells using quantitative RT-PCR. We found that the H1R expression of T cells was significantly increased in the Pd-sensitized group compared with the PBS-sensitized group at 12 and 24 h after the challenge (Fig. 4A). Both CD4⁺ and CD8⁺ T cells have H1R mRNA expression (Fig. 4B). In addition, histamine stimulation increased expression of H1R mRNA in both CD4⁺ and CD8⁺ T cells (Fig. 4C).

3.4. The effects of histamine and OLP *in vitro*

To verify the effects of histamine on activated T cells, we examined the change in IFN- γ production in T cells following stimulation of histamine or of the antihistamine, OLP, *in vitro*. Cells were isolated from splenocytes and B cells were removed, then activated T cells were obtained by culture with IL-2 and anti-CD3/CD28 beads (control). The activated T cells were divided into three stimulation groups, negative control, histamine stimulation, and histamine with OLP stimulation, and IFN- γ production was analyzed using Flow cytometry. Few IFN- γ producing CD4⁺ and CD8⁺ T cells were observed in the absence of histamine stimulation (Fig. 5A); however, after histamine stimulation IFN- γ production was detected in both CD4⁺ and CD8⁺ T cells, particularly CD8⁺ T cells which produced markedly (Fig. 5B). In contrast, treatment

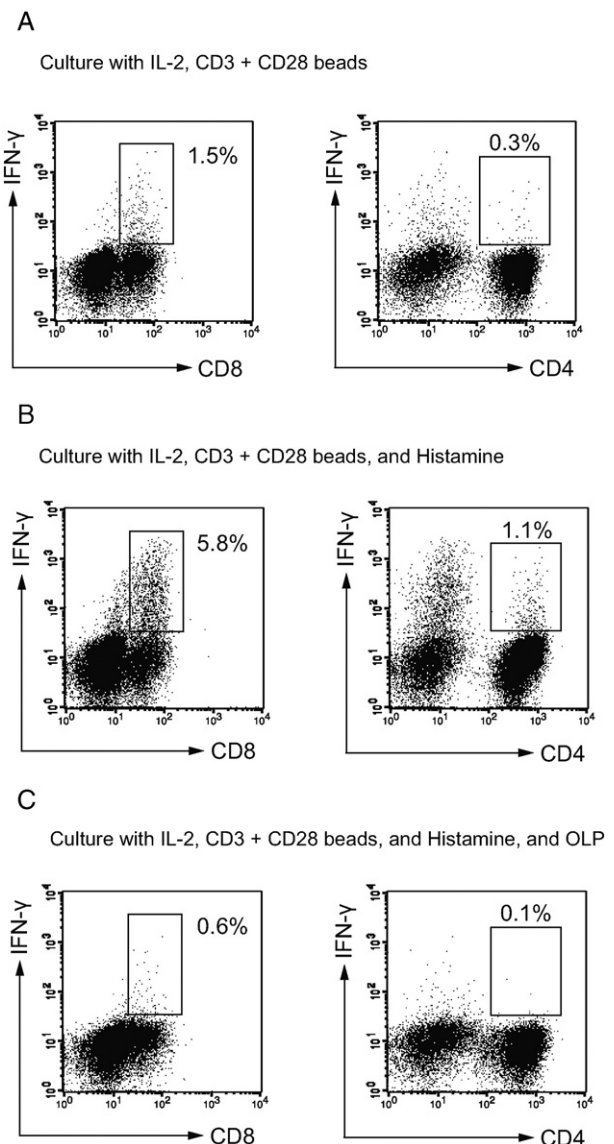


Fig. 5. *In vitro* stimulation of T cells with histamine and olopatadine hydrochloride (OLP). Cells were isolated from splenocytes and B cells were removed prior to culture with rIL-2 and Mouse T-Activator anti-CD3 and anti-CD28 antibody-coated beads for 3 days. For the histamine stimulation group, histamine was added for 24 h (from day 2 to 3 of culture). For the OLP stimulation group, histamine was added for 24 h, as described above, and then OLP was added for 4 h on day 3 of culture. The dot plot shows IFN- γ production by T cells as assessed by Flow cytometry. Percentages were calculated based on setting total T cells as 100%. (A), control group; (B), histamine stimulation group; (C), OLP stimulation group.

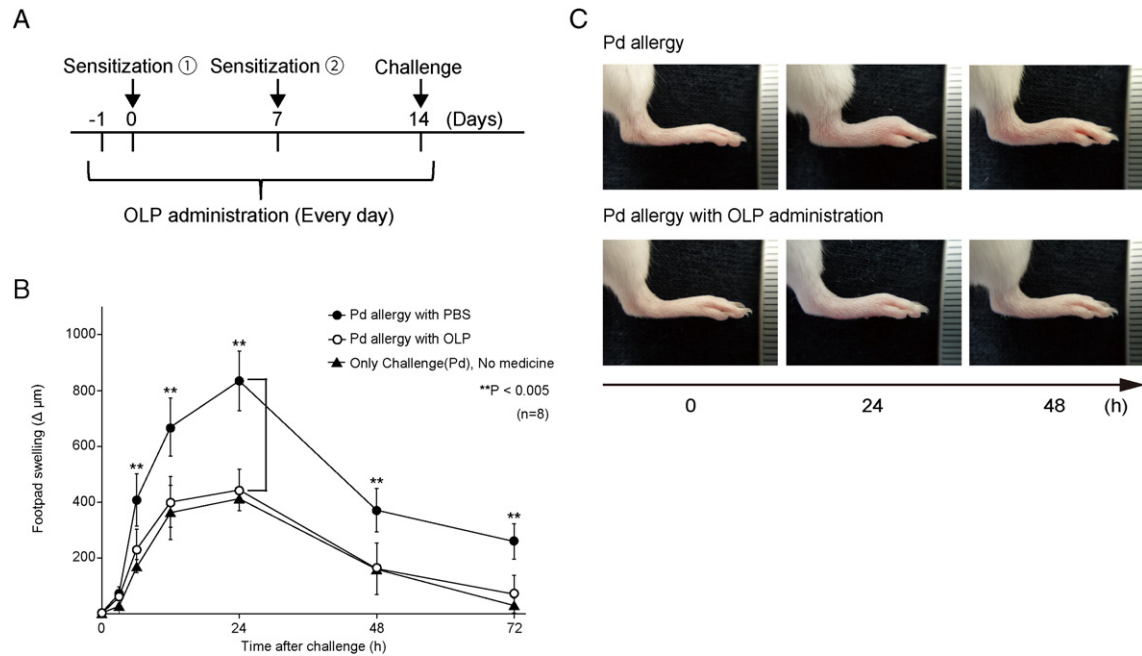


Fig. 6. Effect of olopatadine hydrochloride (OLP) on footpad swelling. (A) Pd allergy was induced in mice as indicated in Fig. 1A. OLP was injected 1 h prior to the sensitization or challenge on the day of sensitization (days 0 and 7) and challenge (day 14). The dose of OLP was 1 mg/kg of weight in 200 μL of PBS. As a control, mice were injected i.p. with PBS instead of OLP. (B) Footpad swelling was measured before and at 3–72 h after the challenge and the difference in footpad thickness before and after challenge was calculated. Closed circles (●) represent Pd allergy mice administered PBS and open circles (○) represent Pd allergy mice administered OLP. Closed triangles (▲) represent sensitized (PBS) and challenged (Pd) mice without administration of OLP. In all groups, footpad swelling peaked at 24 h after Pd challenge; however, in the group administered OLP footpad swelling was markedly suppressed. Data are shown as the mean $\pm$ SD (n = 8). Statistical significance was tested by unpaired Student's *t*-test (** $p < 0.005$). (C) Photographs showing footpad swelling of a representative mouse at 0, 24, and 48 h after Pd challenge. The top row shows footpads from Pd allergy mice and the bottom row shows footpads of mice administered OLP. In both groups the peak in footpad swelling was observed at 24 h after challenge with Pd and there was clear swelling and redness of the footpad, which were suppressed by OLP administration.

with OLP suppressed IFN- γ production in response to histamine in both CD4 $^{+}$ and CD8 $^{+}$ T cells (Fig. 5C).

3.5. OLP suppresses footpad swelling

Next, we examined whether *in vivo* OLP administration could affect the degree of footpad swelling in our metal allergy model. Pd allergy was induced in mice as indicated in Fig. 1A. Mice were injected with OLP once per day on days –1 to 14 (Fig. 6A). Footpad swelling peaked at 24 h after Pd challenge in all groups (Fig. 6B), and was decreased by approximately 50% in the OLP administration group compared with the Pd allergy group. Further, the group administered with OLP responded similarly to the mock-sensitized (PBS) in Pd-challenged mice. As shown in a representative photograph, clear swelling and redness of the footpad were observed at 24 h after Pd challenge, which were suppressed by OLP administration (Fig. 6C).

3.6. OLP suppresses IFN- γ production

To verify the effects of OLP on T cells *in vivo*, we examined whether IFN- γ production is altered in our model. Mice were sacrificed at 15 h after the challenge and lymphocytes were isolated from popliteal lymph node cells followed by analysis of IFN- γ production using Flow cytometry (Fig. 7A). OLP significantly suppressed the production of IFN- γ in CD8 $^{+}$ T cells (Fig. 7B). Thus, this finding further supports the role of histamine in T cell activation during the allergic response to metal.

4. Discussion

In this study we improved the existing Pd allergy mouse model and employed it in order to demonstrate that histamine induces the

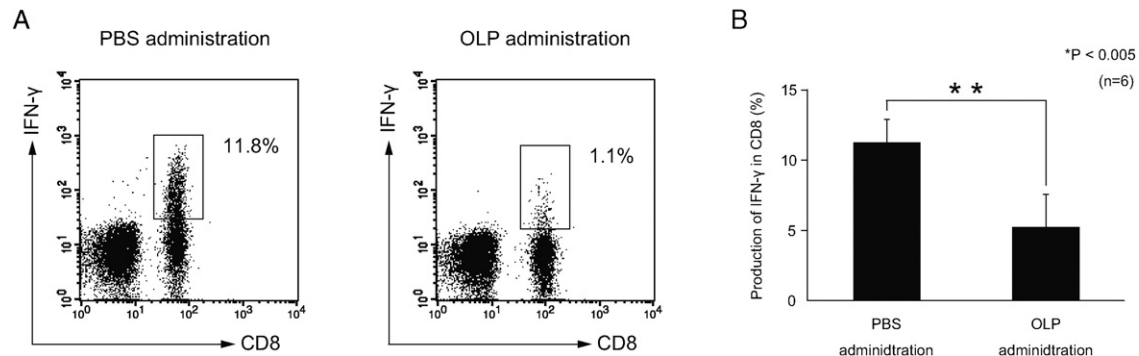


Fig. 7. Effect of OLP on IFN- γ production *in vivo*. Induction of Pd allergy in mice and OLP administration were described in Fig. 6A. Popliteal lymph node cells were isolated at 15 h after Pd challenge, and cytokine production was analyzed using Flow cytometry. (A) IFN- γ production was compared in CD8 $^{+}$ T cells from Pd allergy mice administered PBS or OLP. (B) Statistical analysis of IFN- γ production in CD8 $^{+}$ T cells. Error bars indicate mean $\pm$ SD (n = 6). Statistical significance was tested by unpaired Student's *t*-test (* $p < 0.005$). The percentage was calculated by setting total lymphocytes as 100%.

production of IFN- γ by T cells during the Pd allergic response. Moreover, blockade of H1R by a commonly used antihistamine resulted in suppression of IFN- γ production from T cells and a reduction in Pd allergy symptoms in this model.

Previously, CD4⁺ T cells were reported to be involved in the effector and regulatory functions of the allergic response by releasing IL-4 and IL-10 [21–24]. However, other studies using CD4⁺ and CD8⁺-Ab-depleted mice and MHC classII or classI knockout mice suggested the importance of hapten-specific IFN- γ producing CD8⁺ T cells for the development of the allergic response to dinitrofluorobenzene [21,22,24]. In our Pd allergy model, CD8⁺ T cells were found to be the major producers of IFN- γ as is characteristic of the delayed-type hypersensitivity reaction. While CD4⁺ T cells may also be involved in the Pd allergy, we could not detect IL-4, IL-10, or IL-17 production in our experiments. Thus, together these findings suggest that CD8⁺ T cells mainly have effector functions in Pd allergy [8].

The H1R is expressed in T cells and histamine has been reported to regulate the function of CD4⁺ T cells during immune responses. In this study, we found that histamine promotes H1R expression and production of IFN- γ in CD8⁺ T cells. The importance of H1R for eliciting optimal T cell responses has been shown by H1R^{-/-} mice, which exhibit reduced antigen-specific proliferative responses [25]. Moreover, it was reported that H1R signaling at the time of TCR ligation is required for activation of p38 MAPK, a known regulator of IFN- γ expression [26]. Together, these results suggest that histamine primarily activates CD8⁺ T cells thereby promoting a cell-mediated immune response. In the current study, we showed that histamine promotes Pd allergy symptoms and production of IFN- γ in the Pd allergy model.

We found that OLP suppressed CD8⁺ T cell activity induced by histamine stimulation as well as footpad swelling in Pd allergy mice. In addition, OLP suppressed IFN- γ production both *in vitro* and *in vivo*. Thus, this antihistamine was suggested to effectively suppress the cell-mediated immune response during metal allergy.

In conclusion, we found that histamine induces the production of IFN- γ in T cells, which is suppressed by administration of the antihistamine OLP. Furthermore, in the Pd allergy mouse model footpad swelling was suppressed by OLP. These results suggest that histamine promotes the allergic reaction to Pd and therefore, H1R is an effective therapeutic target for the treatment of metal allergy.

Disclosure

The authors declare that there are no conflicts of interest.

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