JDA Japanese Dermatological Association
THE JOURNAL OF DERMATOLOGY
doi: 10.1111/j.1346-8138.2009.00726.x
Journal of Dermatology 2009; **36**: 654–657

LETTER TO THE EDITOR

Antihistaminic drug olopatadine downmodulates CCL17/TARC production by keratinocytes and Langerhans cells

Dear Editor,

Olopatadine hydrochloride (OLP; [Z]-11-[3-dimethylaminopropylidene]-6,11- dihydrodinenz [b,e] oxepin-2-acetic acid monohydrochloride) is a histamine H_1-receptor-blocking agent that possesses both acidic and basic residues.[1] This H_1 blocker also suppresses the production by epithelial cells or mast cells of various chemical mediators and cytokines, such as leukotrienes, arachidonic acid, interleukin (IL)-6, IL-8 and tumor necrosis factor-α (TNF-α),[2,3] and inhibits intracellular adhesion molecule 1 (CD54) expression on conjunctival cells[4] and activity/migration of eosinophils.[5] Based on these findings, OLP is now widely used for the treatment of allergic rhinitis, urticaria and various itchy skin diseases including eczematous dermatitis.[6]

It has been reported that OLP has a unique anti-allergic property, which may provide implications for the mechanisms underlying its therapeutic actions. Thymus and activation-regulated chemokine (CCL17/TARC) is one of the T-helper (Th)2-associated chemokines, and an important regulator of Th2 cell recruitment into the skin.[7] Serum CCL17 level is proportional to the disease activity of atopic dermatitis (AD), and OLP inhibits CCL17 production by peripheral blood mononuclear cells from AD patients.[7] Serum CCL17 level is also related to the disease activity of bullous pemphigoid, mycosis fungoides, chronic actinic dermatitis and papuloerythroderma.[8,9] In the skin, CCL17 is secreted by keratinocytes (KC) and Langerhans cells (LC). LC are professional antigen-presenting cells in the epidermis, and we have recently shown that they are the main source of CCL17 among epidermal cells.[10] These findings urged us to investigate whether OLP induces inhibition of CCL17 production by KC or LC *in vitro*. To examine the effects of OLP on KC, we used human KC cell line HaCaT cells. LC-enriched epidermal cells (LC-EC) and bone marrow-derived dendritic cells (BMDC) were prepared from BALB/c mice. Our results suggest that OLP exerts its therapeutic effectiveness by inhibiting CCL17 production by both KC and LC.

First, to explore whether olopatadine suppresses CCL17 production by KC, we added olopatadine into the culture medium of KC cell line HaCaT cells. Three-day culture supernatants from HaCaT cells were collected, stored at −80°C and measured for CCL17, CCL22/MDC, monokine induced by γ-interferon (IFN-γ) (CXCL9/Mig) and IFN-γ-inducible protein 10 (CXCL10/IP-10) using enzyme-linked immunosorbent assay (ELISA) kits (Genzyme/Techne, Minneapolis, MN, USA) according to the manufacture's directions. It has been reported that the concentrations of OLP at 10^{-5} to 10^{-7} mol/L suppresses *in vitro* activities of both KC and LC.[11] Therefore, we followed the protocols to examine the inhibitory activity of olopatadine in our experiments. As shown in Figure 1, the IFN-γ/TNF-α-augmented production of CCL17 was suppressed significantly by the addition of olopatadine at a concentration of 10^{-6} or 10^{-5} mol/L. The concentrations of OLP in this *in vitro* study were chosen on the basis of the therapeutic dose of this drug.[12]

To see the effects of olopatadine on CCL17 production by LC-EC, epidermal cell (EC) suspensions freshly isolated from naïve BALB/c mice were subjected to Ficoll gradient separation of LC-EC as described previously.[13] The percentage of LC in LC-EC fraction was 15–20%, as assessed by flow cytometric analysis with anti-I-A^d phycoerythrin (PE)-labeled monoclonal antibody (BD PharMingen, San Diego, CA, USA). OLP

Correspondence: Kazunari Sugita, M.D., Department of Dermatology, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan. Email: k-sugita@med.uoeh-u.ac.jp

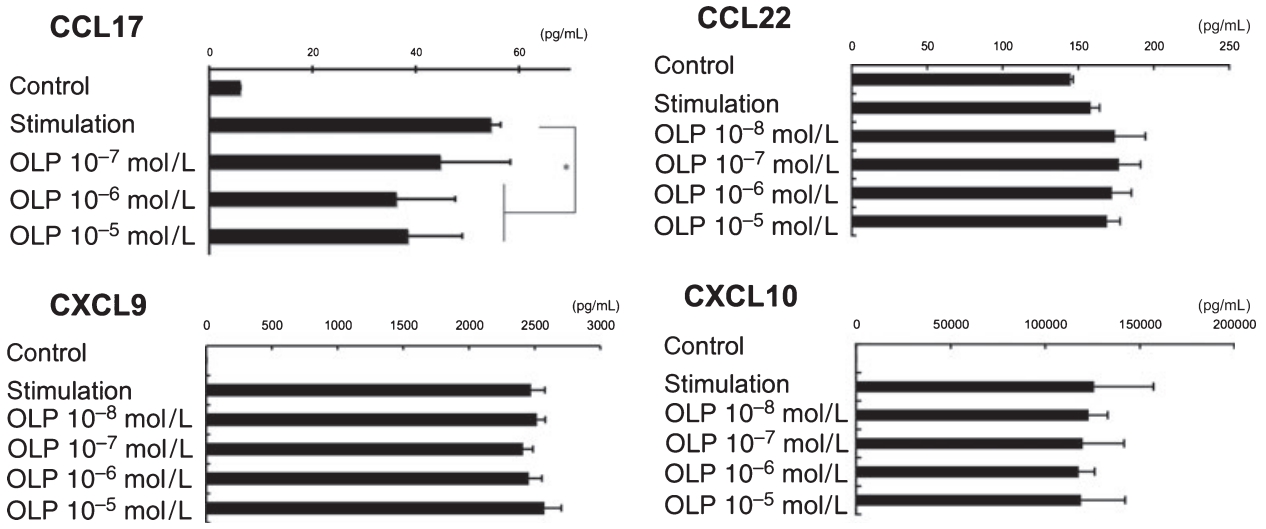

Figure 1. To examine chemokine production, semiconfluent HaCaT cells in 24-well plates were stimulated with 2000 units/mL of recombinant γ-interferon (IFN-γ) (Biogamma; Maruho Pharmaceutical, Osaka, Japan) and 4000 units/mL of tumor necrosis factor-α (TNF-α) (Invitrogen, Carlsbad, CA, USA) for the first 2 h, followed by 200 units/mL IFN-γ and 400 units/mL TNF-α thereafter. Olopatadine (OLP) was added at the starting of culture. Three-day culture supernatants were measured for CCL17, CCL22, CXCL9 and CXCL10 by enzyme-linked immunosorbent assay. Data represent the mean ± standard deviation. $^{*}P < 0.05$.

downregulated the expression of mRNA for CCL17 but not CCL22 (Fig. 2a). Because KC coexist with LC in LC-EC fraction, we also investigated the production of CCL17 by BMDC, a mimicry of pure LC. Murine immature DC were generated from bone marrow according to standard protocols.[14,15] Minor modification included feed culture medium on day 3 containing granulocyte-macrophage colony-stimulating factor (10 ng/mL). On day 6, BMDC (5×10^6/well) were cultured for 24 h with the two indicated concentrations of OLP. As shown in Figure 2(b), OLP decreased the mRNA expression of CCL17 and CCL22 in mature BMDC. Three independent series of experiments confirmed the result. CCL17 in culture supernatants was quantified by ELISA. OLP significantly suppressed the production of CCL17 by 37%, while the production of CCL22, CXCL9 or CXCL10 was not inhibited (data not shown). The above findings suggested that OLP directly downregulates Th2 chemokine production by DC and LC.

It has been reported that PAM 212 cells, a murine KC cell line, and normal human KC produce CCL17 after stimulation with TNF-α and IFN-γ.[16,17] Consistent with these *in vitro* data, CCL17 is expressed in the lesional KC of AD skin, suggesting that KC is one of the main sources of CCL17.[17] CCL17-transgenic mice showed enhanced Th2 type contact hypersensitivity and reduced Th1 type reactivity.[18] In this study, we demonstrated that OLP downmodulates the production of CCL17 by epidermal KC.

We have previously demonstrated that the ability of LC to present hapten to prime T cells was reduced by OLP with decreased expression of major histocompatibility complex class II and co-stimulatory molecules.[11] LC are capable of producing a high level of CCL17 constitutively during culture even without exogenous stimuli,[19] and we have recently shown that LC are responsible for the production of CCL17 by epidermal cells.[10] The present study showed that OLP inhibits the production of CCL17 by LC-EC. Thus, OLP is effective for the treatment of Th2-associated skin disorders not only by suppressing antigen-presenting ability but also by inhibiting CCL17 production. In our experiment system using LC-EC, KC coexisted with LC, raising the possibility that OLP alters CCL17 production by LC indirectly by modulating bystander KC. Therefore, another DC population without contamination of KC was tested for the modulatory effect of OLP on the chemokine production. Because Th2 chemokines including CCL17 was

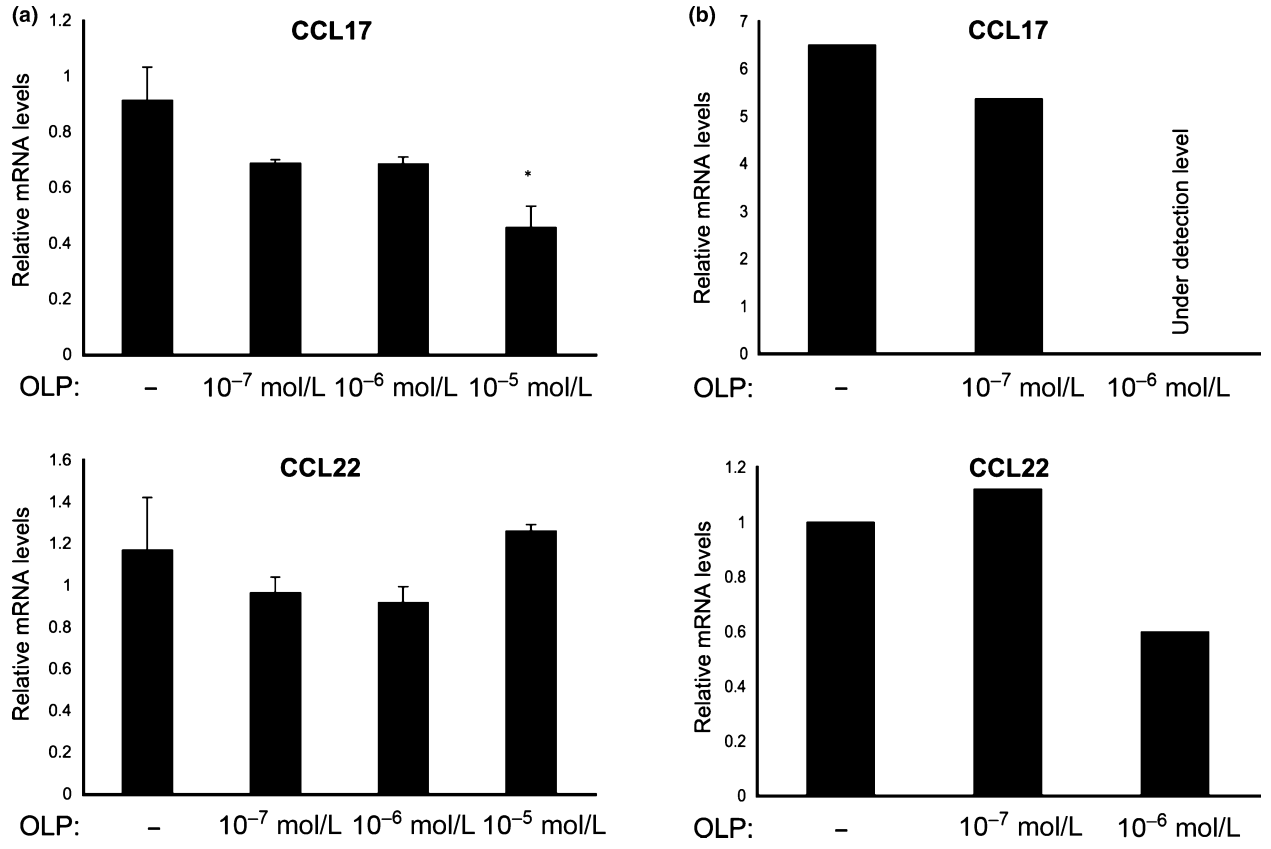

Figure 2. Total cellular RNA was extracted with an RNA extraction kit (Promega, Madison, WI, USA) from cultured Langerhans cell-enriched epidermal cells (LC-EC) and bone marrow-derived dendritic cells (BMDC). RNA was then reverse-transcribed and amplified by random hexamer in single-tube assay using the TaqMan Reverse Transcription Reagants (Applied Biosystems, Foster City, CA, USA) with gene-specific sense and antisense primers and a detection probe labeled on the 5′-end with the reporter dye 6-FAM. Primers and probes were obtained from TaqMan Gene Expression Assays Inventories (accession numbers: CCL17, Mm00516136-m1; CCL22, Mm00436439-m1; β-actin, 4352933E; all for Applied Biosystems). Using an ABI Prism 7000 Sequence Detection Systems (Applied Biosystems), samples were reverse-transcribed and amplified. Quantification of gene-specific message levels was determined by comparing fluorescence intensity from unknown RNA samples to the fluorescence intensity of standard curve generated from control mRNA levels. Amplification of the gene for mouse β-actin was performed on all samples to control interspecimen variations in RNA amounts. (a) mRNA expression for chemokines in LC-EC. LC-EC from naïve mice were cultured with or without olopatadine for 24 h. The cultured cells were subjected to real-time polymerase chain reaction analysis for CCL17 and CCL22. Data are expressed as the mean ± standard deviation of triplicate culture. *$P < 0.05$, compared with the olopatadine non-added one. (b) mRNA expression for chemokines in BMDC. BMDC were cultured for 24 h with or without olopatadine. The cultured cells were subjected to real-time polymerase chain reaction analysis for CCL17 and CCL22. The data are from a representative experiment out of three.

expressed in a subset of BMDC, we investigated the effect of OLP on CCL17 production by BMDC. OLP downregulated both CCL17 and CCL22 production by BMDC. Besides the effects of OLP on KC and LC, another study has shown that antihistamines regulate immune responses by affecting the interaction between DC and $CD4^+$ T cells.[20]

In summary, OLP suppresses the production of CCL17 by KC and DC. This suggests that OLP may exerts its therapeutic effect at least partly by down-modulating Th2 chemokine production by epidermal cells.

Kazunari SUGITA, Miwa KOBAYASHI,
Tomoko MORI, Kenji KABASHIMA,
Motonobu NAKAMURA, Yoshiki TOKURA
Department of Dermatology,
University of Occupational and Environmental Health,
Kitakyushu, Japan

CONFLICT OF INTEREST

The authors declare that this study was financially supported in part by Kyowa Engineering Co., Ltd.

REFERENCES

1 Ohmori K, Ishii H, Sasaki Y, Ikemura T, Manabe H, Kitamura S. Effects of KW-4679, a new orally active antiallergic drug, on antigen-induced bronchial hyperresponsiveness, airway inflammation and immediate and late asthmatic responses in guinea pigs. *Int Arch Allergy Immunol* 1996; **110**: 64–72.

2 Yanni JM, Weimer LK, Sharif NA, Xu SX, Gamache DA, Spellman JM. Inhibition of histamine-induced human conjunctival epithelial cell responses by ocular allergy drugs. *Arch Ophthalmol* 1999; **117**: 643–647.

3 Cook EB, Stahl JL, Barney NP, Graziano FM. Olopatadine inhibits TNF-α release from human conjunctival mast cells. *Ann Allergy Asthma Immunol* 2000; **84**: 504–508.

4 Cook EB, Stahl JL, Barney NP, Graziano FM. Olopatadine inhibits anti-immunoglobulin E-stimulated conjunctival mast cell upregulation of ICAM-1 expression on conjunctival epithelial cells. *Ann Allergy Asthma Immunol* 2001; **87**: 424–429.

5 Ikemura T, Manabe H, Sasaki Y *et al.* KW-4679, an antiallergic drug, inhibits the production of inflammatory lipids in human polymorphonuclear leukocytes and guinea pig eosinophils. *Int Arch Allergy Immunol* 1996; **110**: 57–63.

6 Ohmori K, Hasegawa K, Tamura T *et al.* Properties of olopatadine hydrochloride, a new antiallergic/antihistaminic drug. *Arzneimittelforschung* 2004; **54**: 809–829.

7 Furukawa H, Takahashi M, Nakamura K, Kaneko F. Effect of an antiallergic drug (Olopatadine hydrochloride) on TARC/CCL17 and MDC/CCL22 production by PBMCs from patients with atopic dermatitis. *J Dermatol Sci* 2004; **36**: 165–172.

8 Tamaki K, Kakinuma T, Saeki H *et al.* Serum levels of CCL17/TARC in various skin diseases. *J Dermatol* 2006; **33**: 300–302.

9 Shimauchi T, Sugita K, Nishio D *et al.* Alterations of serum Th1 and Th2 chemokines by combination therapy of interferon-γ and narrowband UVB in patients with mycosis fungoides. *J Dermatol Sci* 2008; **50**: 217–225.

10 Mori T, Kabashima K, Yoshiki R *et al.* Cutaneous hypersensitivities to hapten are controlled by IFN-γ-upregulated keratinocyte Th1 chemokines and IFN-γ -downregulated langerhans cell Th2 chemokines. *J Invest Dermatol* 2008; **128**: 1719–1727.

11 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.

12 Tsunoo M, Momomura S, Masuo M *et al.* Phase I clinical study on KW-4679, an antiallergic drug. *Kiso To Rinsho* 1995; **29**: 93–111.

13 Sugita K, Kabashima K, Atarashi K, Shimauchi T, Kobayashi M, Tokura Y. Innate immunity mediated by epidermal keratinocytes promotes acquired immunity involving Langerhans cells and T cells in the skin. *Clin Exp Immunol* 2007; **147**: 176–183.

14 Inaba K, Inaba M, Romani N *et al.* Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J Exp Med* 1992; **176**: 1693–1702.

15 Vabulas RM, Braedel S, Hilf N *et al.* The endoplasmic reticulum-resident heat shock protein Gp96 activates dendritic cells via the Toll-like receptor 2/4 pathway. *J Biol Chem* 2002; **277**: 20847–20853.

16 Vestergaard C, Bang K, Gesser B, Yoneyama H, Matsushima K, Larsen CG. A Th2 chemokine, TARC, produced by keratinocytes may recruit CLA+CCR4+ lymphocytes into lesional atopic dermatitis skin. *J Invest Dermatol* 2000; **115**: 640–646.

17 Vestergaard C, Yoneyama H, Murai M *et al.* Overproduction of Th2-specific chemokines in NC/Nga mice exhibiting atopic dermatitis-like lesions. *J Clin Invest* 1999; **104**: 1097–1105.

18 Saeki H, Tamaki K. Thymus and activation regulated chemokine (TARC)/CCL17 and skin diseases. *J Dermatol Sci* 2006; **43**: 75–84.

19 Fujita H, Asahina A, Sugaya M *et al.* Differential production of Th1- and Th2-type chemokines by mouse Langerhans cells and splenic dendritic cells. *J Invest Dermatol* 2005; **124**: 343–350.

20 Iida H, Asada H, Yokoi S *et al.* Regulatory effects of antihistamines on the responses to staphylococcal enterotoxin B of human monocyte-derived dendritic cells and CD4+ T cells. *J Dermatol Sci* 2008; **52**: 31–38.

