

Letter to the Editor

Antihistaminic drug olopatadine downmodulates T cell chemotaxis toward CCL17 in patients with atopic dermatitis



Dear Editor

Olopatadine hydrochloride (OLP) is an antihistaminic drug with selective histamine H1 receptor antagonist activity.¹ OLP has inhibitory effects on the production of various chemical mediators and cytokines, such as interleukin (IL)-6, IL-8, and tumor necrosis factor- α (TNF- α).^{2,3} It inhibits TNF- α release from human conjunctival mast cells, and consequently decreases CD54 expression on the cells.³ In the skin, the ability of Langerhans cells (LCs), epidermal antigen-presenting dendritic cells (DCs), to present hapten to T cells is reduced by OLP with decreased expression of major histocompatibility complex class II and co-stimulatory molecules.⁴ Furthermore, OLP downmodulates CCL17/TARC production by LCs as well as keratinocytes (KCs), as OLP at a concentration of 10^{-6} or 10^{-5} mol/L suppresses the IFN- γ /TNF- α -augmented production of CCL17 from HaCaT cells and the mRNA expression of CCL17 and CCL22 in mature bone marrow DCs, a mimicry of pure LCs.⁵ More recently, OLP was reported to decrease tissue IL-31 and thymic stromal lymphopoietin levels in an atopic dermatitis (AD) mouse model.^{6,7} An *in vivo* study also showed decreased serum levels of CCL17 and CCL22 in AD patients after treatment with OLP.⁸ These findings suggest that OLP downmodulates Th2-mediated skin inflammation at least in part by interfering with the antigen-presenting and chemokine-producing abilities of DCs. However, it remains to be elucidated whether OLP directly regulate the action of Th2 cells. In this study, we thus studied the effect of OLP on T cell chemotactic activity toward the Th2 chemokine, CCL17.

The study was approved by the ethical committee of Hamamatsu University School of Medicine. Seven patients with AD who had not been treated with oral antihistaminic or immunosuppressive drugs were enrolled in this study after providing their informed consent. CD4⁺ T cells were isolated from peripheral blood mononuclear cells (PBMCs) by negative selection using a CD4⁺ T Cell Isolation Kit II (Miltenyi Biotec, Auburn, CA, USA) and complete RPMI-1640 was used as the culture medium.⁹ Cells were incubated with or without 1×10^{-6} M or 1×10^{-5} M OLP hydrochloride (Kyowa-Kirin, Tokyo, Japan) in complete RPMI-1640 for 8 h. Actually, the concentration of 1×10^{-5} M is rather

high than the actual concentration of Cmax in serum by intake of 10 mg olopatadine chloride. However, 1×10^{-6} M is nearly actual concentration of Cmax. The cell viabilities had been confirmed by Trypan blue staining, that showed no toxic effects of 1×10^{-6} and 1×10^{-5} M olopatadine. Time-lapse images of cell migration during chemotaxis were observed directly using EZ-TAXIScan, an optically accessible, horizontal real-time chemotaxis analyzer, via a charge-coupled device (CCD) camera (GE Healthcare, Tokyo, Japan). The migration traces of CD4⁺ T cells were plotted against time and distance, and velocity was calculated by ImageJ software (National Institutes of Health, Bethesda, Maryland, USA).

As shown in Fig. 1a, representative chemotaxis data with CD4⁺ T cells from an AD patient demonstrated that CD4⁺ T cells without OLP incubation migrated towards CCL17 in a straightforward manner (black lines). However, cells incubated with 1×10^{-5} M OLP (blue lines) exhibited random movement. Treatment with 1×10^{-6} M OLP yielded moderately random movement (red lines). In all 7 patients, the mean velocity levels of CD4⁺ T cells toward CCL17 were significantly suppressed by 1×10^{-5} M OLP compared with non-treated controls (Fig. 1b) ($P = 0.001$ compared with the non-treated group). Pearson's correlation test has been demonstrated between TARC and Velocity. The correlation coefficient is 0.97 that means strong positive correlation. In addition, the olopatadine effectively inhibited chemoattraction of CD4⁺ T cells toward TARC in the patient with lower serum TARC. These figures were added as the supplemental data (Supplementary Fig. 1a, b). Olopatadine also inhibits the activity of CD4⁺ T cell chemoattractant from healthy control more strongly toward TARC compared to AD patients. The representative result has been added as the supplemental data (Supplementary Fig. 2).

CCL17 is a ligand for the CCR4 chemokine receptor, and the expression of CCR4 on T cells is increased in patients with AD.¹⁰ Therefore, we examined the modulatory effect of OLP on CCR4 expression in CD4⁺ T cells from 7 AD patients. The cell surface expression of CCR4 on CD4⁺ T cells was determined by flow cytometric analysis using FACSCalibur (Becton Dickinson).⁹ The mean fluorescent intensity (MFI) levels of CCR4 on CD4⁺ T cells were moderately but significantly decreased after incubation with OLP at 1×10^{-6} M or 1×10^{-5} M as compared with the non-treated group ($P = 0.03$) (Fig. 2a).

F-actin plays a crucial role in T cell chemotactic movement. To test whether OLP affects F-actin polymerization, flow cytometric

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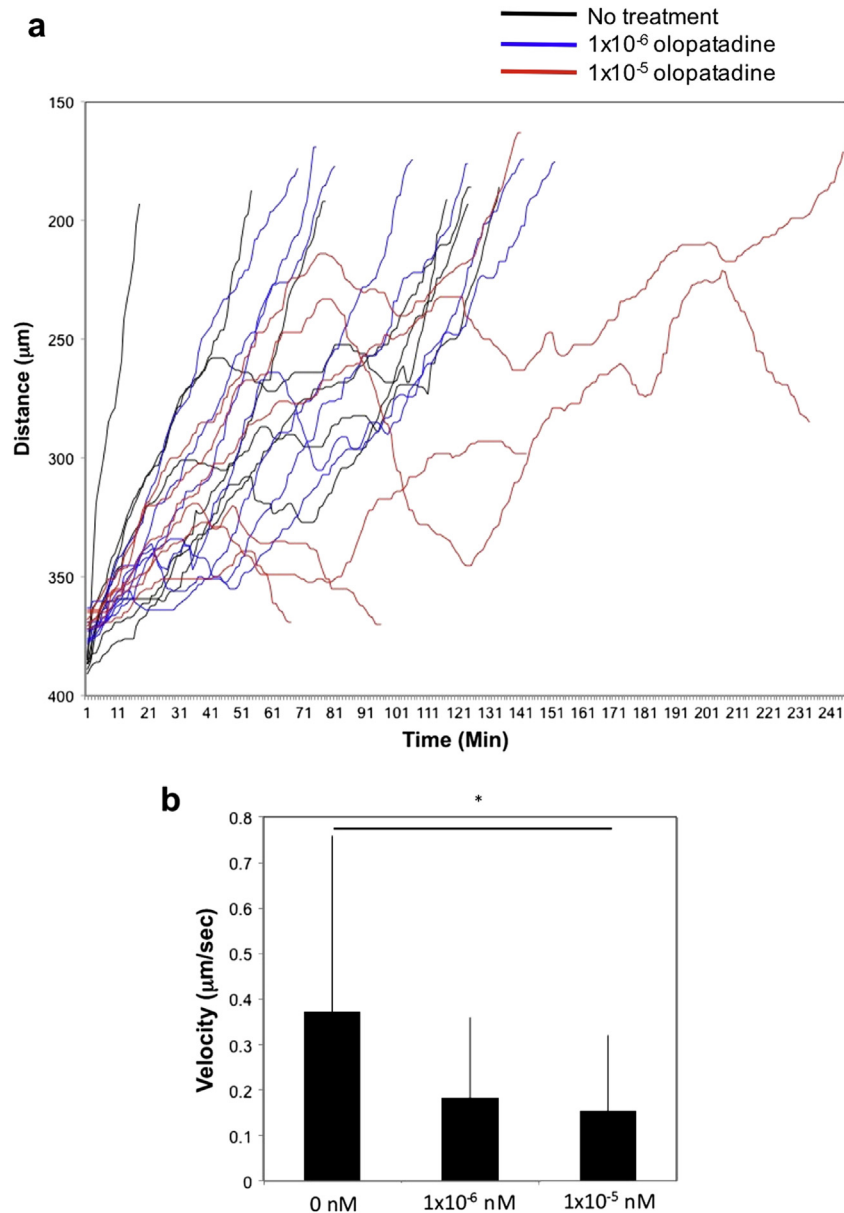


Fig. 1. OLP decreases velocities of $CD4^{+}$ T cells toward CCL17/TARC. The velocities of $CD4^{+}$ T cells from a representative AD patient are shown (a). The mean velocities of $CD4^{+}$ T cells in 7 AD patients are statistically analyzed with Dunnett's method (b). * $P < 0.01$.

analysis of T cells from an AD patient was performed. Since phalloidin binds to polymeric and oligomeric forms of actin, but not to monomeric actin, the level of polymerized actin was determined by staining cells with phalloidin.¹¹ The MFIs of phalloidin were lower in $CD4^{+}$ T cells treated with 1×10^{-5} OLP than in non-treated cells (Fig. 2b), indicating inhibition of F-actin polymerization by OLP.

In this study, we found that OLP downmodulates not only the CCL17/TARC production by LCs and HaCaT KCs, but also the chemoattractive activity of Th2 cells towards CCL17. These two modulatory actions of OLP concerning infiltration of Th2 cells may lead to suppression of inflammatory events occurring in the AD lesion.

The inhibitory effects of OLP may be exerted *via* the downregulation of CCR4 expression and the hindrance of F-actin polymerization. Serum CCL17/TARC level is regarded as a biomonitoring marker that reflects changes in the skin condition of AD more accurately and sensitively than conventional laboratory measures.¹² Serum CCL17/TARC concentration significantly decreased after effective treatment in parallel with the improvement of clinical symptoms, SCORAD index and pruritus score in AD patients.¹³ In addition to its suppressive effects on Th2 cells, we have reported that OLP is a downmodulator of CXCL10-induced Th1/Tc1 chemoattraction.⁹ Our studies suggest that some of antihistamines have anti-allergic action even on T cell chemotaxis.

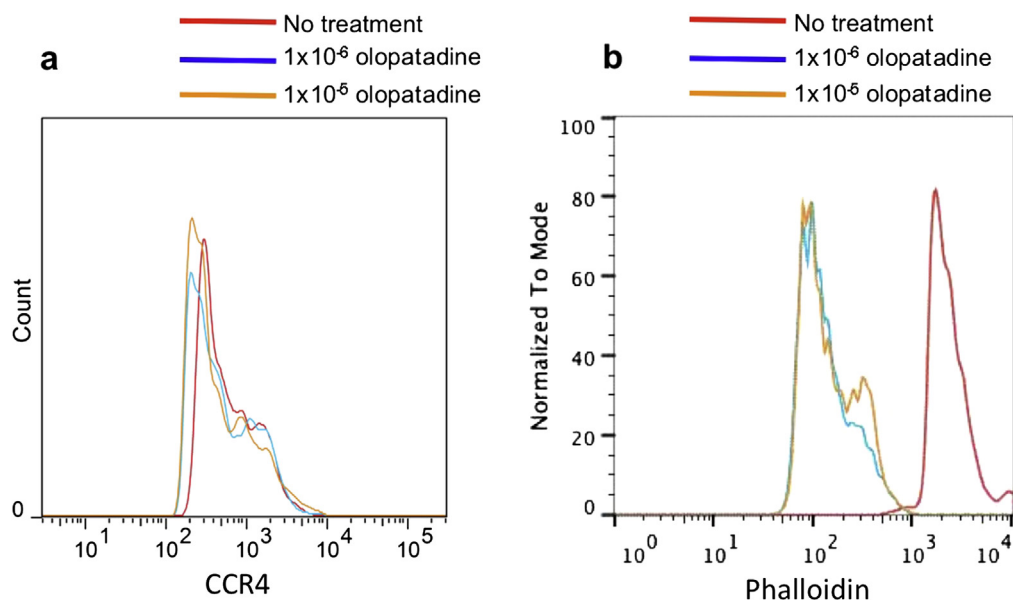


Fig. 2. CCR4 MFI of CD4⁺ T cells from an AD patient (a) and the levels of F-actin, polymerization (b).

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Conflict of interest

The authors have no conflict of interest to declare.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.alit.2014.10.008>.

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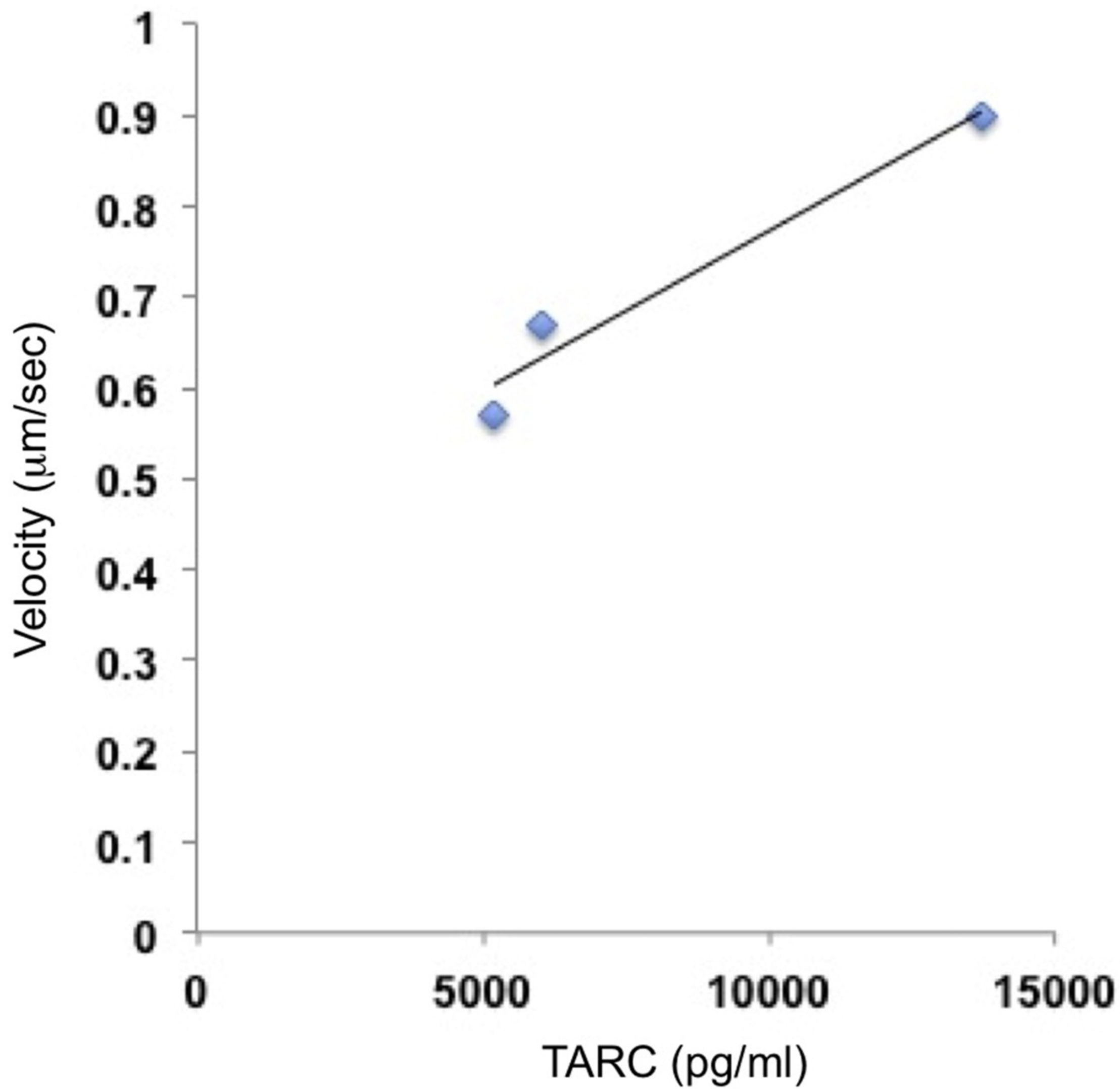
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