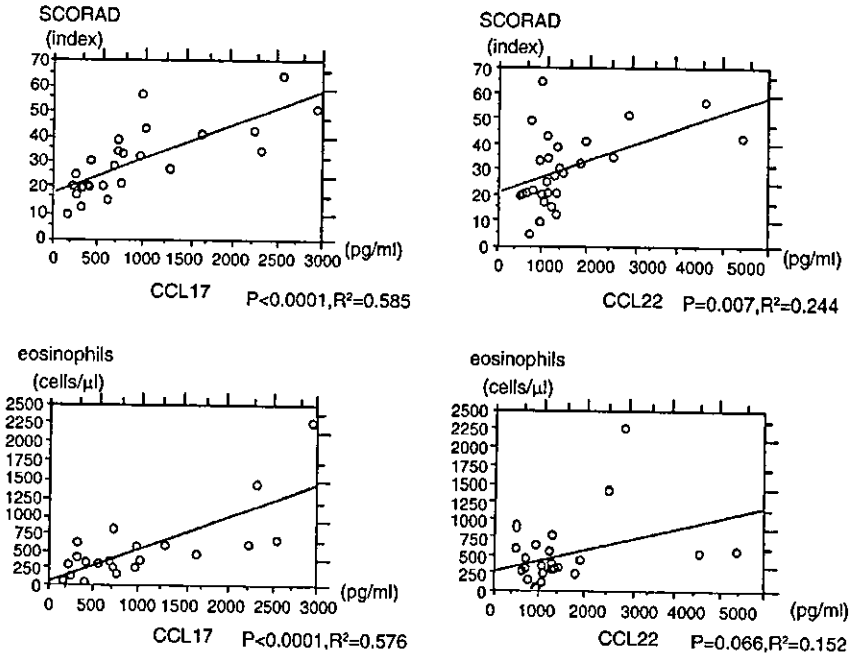

Fig. 1 Correlation between SCORAD index, peripheral blood eosinophil numbers and CCL17 and CCL22 levels in the plasma in AD. Correlation between SCORAD index, and peripheral blood eosinophil numbers was examined, and plasma CCL17 as well as CCL22 levels were measured using ELISA kits, respectively.

treatment and cultured for 3 days with DME tended to be lower than in those from cells taken before the treatment (504.1 ± 101.0 versus 845.9 ± 118.9) (p= 0.116), and were significantly lower when the cells were cultured with DME for 5 days (889.4 ± 232.9 versus 1780.5 ± 205.1) (p = 0.018). CCL17 levels in the supernatants were significantly lower when PBMCs were cultured without DME (Fig. 2).

CCL22 level in the supernatants of PBMCs, taken after the treatment and cultured with DME for 3

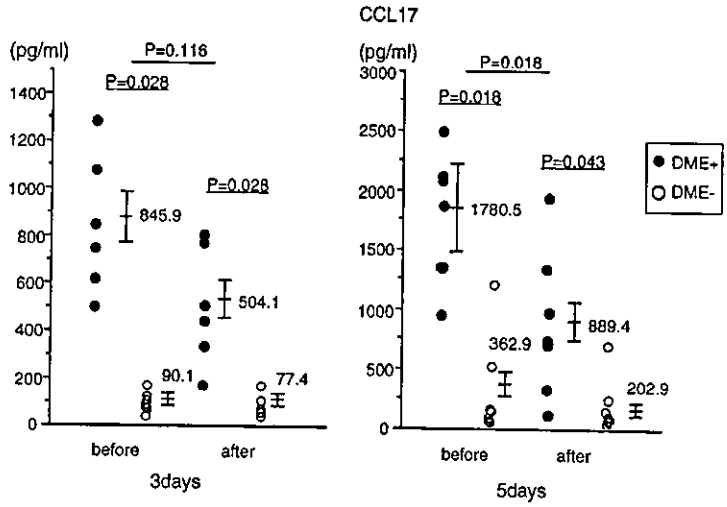

Fig. 2 CCL17 levels in the supernatants of cultured PBMCs taken before and after the treatment with oral Olopatadine. PBMCs were cultured at 37 °C for 3 or 5 days with or without DME (10 μg/ml) stimulation. The levels of CCL17 were measured using ELISA kits. CCL17 levels in the supernatants of cells taken after Olopatadine treatment and cultured for 3 days with DME tended to be lower than in those from cells taken before the treatment, and were significantly lower when the cells were cultured with DME for 5 days.

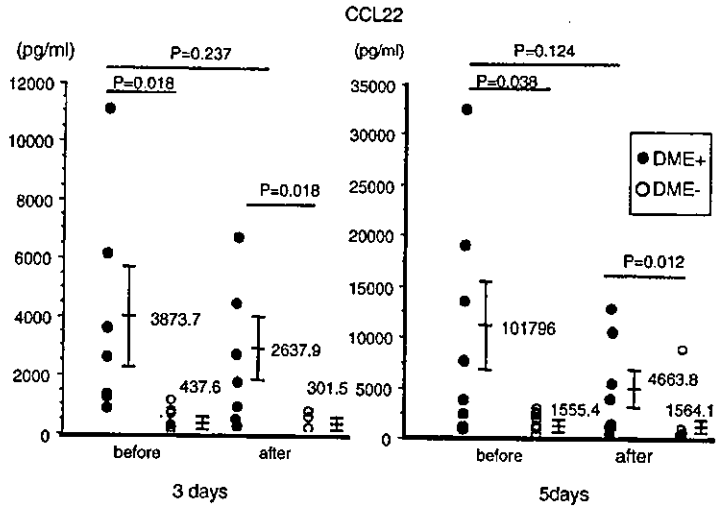

Fig. 3 CCL22 production by cultured PBMCs before and after the treatment with oral Olopatadine. The levels of CCL22 in the supernatants of cultured PBMCs were measured using ELISA kits. CCL22 level in the supernatants of PBMCs, taken after the treatment and cultured with DME for 3 days, tended to be lower than in those from cells taken before the treatment, and the same was true for cells cultured with DME for 5 days.

days, tended to be lower than in those from cells taken before the treatment (2637.9 ± 895.0 versus 3873.7 ± 1391.0) ($p = 0.237$) and the same was true for cells cultured with DME for 5 days (4663.8 ± 1659.0 versus 10179.6 ± 3921.1) ($p = 0.124$). CCL22 levels in the supernatants were significantly lower when cells were cultured without DME (Fig. 3).

There were relatively low levels of IFNγ in the supernatant of cultured PBMCs. IFNγ levels in the supernatants of cultured PBMCs, taken after the treatment and cultured for 5 days with DME were significantly lower than in those from cells taken before the treatment (26.5 ± 5.4 versus 38.3 ± 9.6) ($p = 0.012$). IFNγ levels in the supernatant of PBMCs, taken after the treatment did not show any significant differences compare to those taken before the treatment when the cells were cultured without DME (Fig. 4).

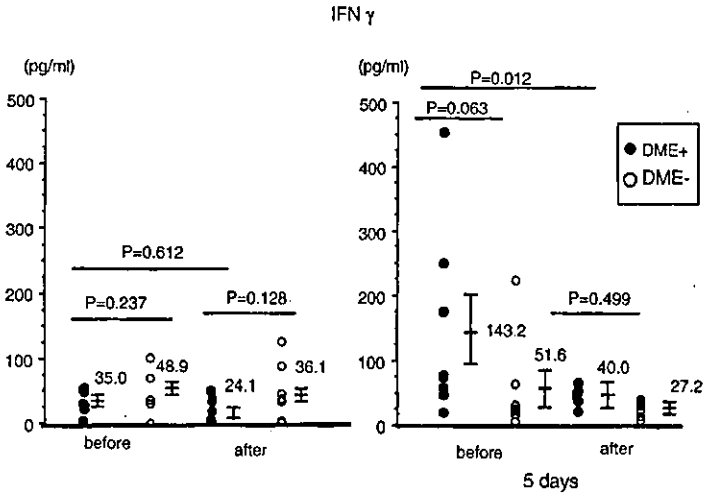

Fig. 4 IFNγ production by cultured PBMCs taken before and after the treatment with oral Olopatadine. The levels of IFNγ in the supernatants of cultured PBMCs were measured using ELISA kits. IFNγ levels in the supernatants of cultured PBMCs, taken after the treatment and cultured for 5 days with DME were significantly lower than in those from cells taken before the treatment.

Table 2 CCL17, CCL22, IFNγ, IL-12 and IL-18 levels in the supernatants of cultured PBMCs for 3 days which were taken before and after the treatment with oral Olopatadine

		Before	After	p
CCL17 (pg/ml)	DME+	845.9 ± 118.9	504.1 ± 100.9	0.116
	DME−	90.1 ± 16.0	77.5 ± 19.3	0.116
CCL22 (pg/ml)	DME+	3873.7 ± 1391.0	2637.9 ± 894.9	0.237
	DME−	457.6 ± 151.3	301.5 ± 97.6	0.063
IL-12 (pg/ml)	DME+	4.6 ± 0.5	5.1 ± 1.1	0.593
	DME−	6.1 ± 1.1	4.8 ± 0.4	0.109
IL-18 (pg/ml)	DME+	64.5 ± 8.9	54.9 ± 7.5	0.173
	DME−	75.0 ± 29.0	90.8 ± 28.9	0.463
IFNγ (pg/ml)	DME+	35.8 ± 8.2	35.9 ± 13.2	0.917
	DME−	19.2 ± 7.1	24.7 ± 11.8	0.345

PBMCs were cultured at 37 °C for 3 days with or without DME (10 mg/ml) stimulation. The levels of CCL17, CCL22, IFNγ, IL-12 and IL-18 in the supernatants of cultured PBMCs were measured using ELISA kits.

Table 3 Comparison of CCL17, CCL22, IFNγ, IL-12 and IL-18 levels in the supernatants of cultured PBMCs for 5 days, which were taken before and after the treatment with oral Olopatadine

		Before	After	p
CCL17 (pg/ml)	DME+	1780.5 ± 205.1	889.4 ± 232.9	0.018
	DME−	363.0 ± 159.4	202.9 ± 87.3	0.612
CCL22 (pg/ml)	DME+	10179.6 ± 3921.1	4663.8 ± 1658.8	0.124
	DME−	1555.4 ± 356.7	1564.1 ± 1052.7	0.327
IL-12 (pg/ml)	DME+	6.1 ± 1.1	4.9 ± 0.4	0.285
	DME−	6.3 ± 1.2	5.0 ± 0.4	0.285
IL-18 (pg/ml)	DME+	73.5 ± 14.7	66.8 ± 5.8	0.889
	DME−	64.2 ± 16.7	67.9 ± 17.11	0.866
IFNγ (pg/ml)	DME+	143.2 ± 51.5	51.6 ± 25.5	0.012
	DME−	40.0 ± 8.3	27.2 ± 4.6	0.063

The levels of CCL17, CCL22, IFNγ, IL-12 and IL-18 in the supernatants of cultured PBMCs were measured using ELISA kits.

IL-12 and IL-18 levels in the supernatants of PBMCs, which were taken before or after the treatment and cultured with or without DME were not significantly different (Tables 2 and 3).

4. Discussion

TARC/CCL17 and MDC/CCL22 are Th2 chemokines, which bind and attract $CCR4^+$ Th2 type T cells. We reported that there are high levels of CCL17 and CCL22 in the sera of AD patients and that levels were significantly correlated with the severity of AD [14,19].

Olopatadine has an function against the histamine H_1 receptor [3]. It also inhibits the release of chemical mediators [4], expression of adhesion molecules on endothelial cells [5,6], and secretion of neuropeptide [7]. However, the effect of Olopatadine on production of chemokine such as CCL17 and CCL22 by PBMCs of AD patients has not been well elucidated.

In this study, we tried to examine the effects of Olopatadine on CCL17 and CCL22 production by PBMCs from AD patients when used in combination with topical corticosteroids. The SCORAD index, numbers of eosinophil in peripheral blood and levels of plasma CCL17 and CCL22 decreased significantly after treatment with oral Olopatadine for 4 weeks. There was a significant correlation between SCORAD index and plasma levels of CCL17 and CCL22. Because the class and topical amounts of topical corticosteroids per day were not altered between before and after the treatment, we think that the

change of cytokine and ckemokine production before and after the treatment reflect the effect of oral administration of Olopatadine. Our data showed that Olopatadine decreased plasma levels of CCL17 and CCL22 in accordance with the improvement of AD.

Furthermore, CCL17 levels in the supernatants of PBMCs taken after the treatment with Olopatadine and cultured for 5 days with DME, were significantly lower than in those from cells taken before the treatment ($p = 0.018$). CCL17 levels in supernatants of PBMCs cultured for 3 days and CCL22 levels in supernatants from both 3 and 5 days cultures with DME, tended to be lower with PBMCs taken after the treatment than with those taken before treatment. These data indicate that Olopatadine suppresses CCL17 production by PBMCs of AD patients cultured in vitro. We detect dramatic decrease of plasma levels of CCL22/MDC after the treatment. However, MDC production in in vitro culture system is not significantly different before and after the treatment. Further examination will be required to evaluate this difference. One possible reason is that Olopatadine may act not only on PBMCs but also other cells which can produce CCL22/MDC in vivo [23].

We previously showed that keratinocytes, T cells and dendritic cells (DCs) are major sources of CCL17 producing cells in AD patients [14,24]. Our present data show that Olopatadine directly affects PBMCs, such as T cells and DCs, and inhibits their CCL17 and CCL22 production. Although the exact mechanism is not clear yet, one possible mechanism for this is the direct effect of Olopatadine on DCs function. It has been reported that Olopatadine binds specifically to S100 proteins. They belong to intracellular regulatory proteins, undergo a conformational change by calcium-binding, and consequently interact with their target proteins [25]. Thus, we speculate that Olopatadine may have some roles in regulating DCs function. We also found that IFNγ production by PBMCs taken after the treatment with Olopatadine and cultured for 5 days with DME stimulation, was lower than by PBMCs taken before the treatment although no differences were observed when the cells were cultured for 3 days (Table 3). It has been reported that Th1 cytokine mRNAs such as IL-12 participate in chronic AD lesions [26]. Our data suggest that productions of Th1 cytokines may be also influenced by Olopatadine in an in vitro culture system.

We conclude that the production of Th2 chemokines, CCL17 and CCL22, by PBMCs, is dramatically inhibited by Olopatadine administration. These findings should be useful for understanding the inhibitory effect of Olopatadine on cytokine production in the treatment of AD.

References

[1] Leung DYM. Atopic dermatitis: new insights and opportunities for therapeutic intervention. J Allergy Clin Immunol 2000;105:860–76.

[2] Furue M, Ohtsuki M, Ogata F, Ishibashi Y. Responsiveness to interleukin 4 and interleukin 2 of peripheral blood cells in atopic dermatitis. J Invest Dermatol 1991;96: 468–72.

[3] Ohmori K, Hayashi K, Kaise T, et al. Pharmacological,pharmacokinetic and clinical properties of Olopatadine hydrochloride, a new antiallergic drug. Jpn J Pharmacol 2002;88:379–97.

[4] 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.

[5] Ichiro M, Akira K, So O, et al. Histamine enhanced the TNF-α-induced expression of E-selectin and ICAM-1 on vascular endothelial cells. Cell Immunol 1996;171:285–8.

[6] Fukushi N, Matsuhisa M, Shimono T, et al. Inhibitory effect of Olopatadine on antigen-induced eosinophil infiltration and the LFA-1 and Mac-1 expression in eosinophils. Jpn J Pharmacol 2002;88:463–6.

[7] Ikemura T, Sasaki Y, Ishii H, et al. Inhibitory effect of KW-4679, an anti-allergic drug, on tachykinin-mediated airway response induced by electrical vagal stimulation in guinea pigs. Jpn J Pharmacol 1996;71:179–82.

[8] John M, Lori K, Najam A, et al. Inhibition of histamine-induced human conjuntival epithelial cell responses by ocular allergy drugs. Arch Ophathalmol 1999;117:643–7.

[9] Rossi D, Zlotnik A. The biology of chemokines and their receptors. Annu Rev Immunol 2000;18:217–42.

[10] Zlontik A, Yoshie O. Chemokines: a new classification system and their role in immunity. Immunity 2000;12:121–7.

[11] Galli G, Chantry D, Annunziato F, Romagnani P, Cosmi L, Lazziri E, et al. Macrophage-derived chemokine production by activated human T cells in vitro and in vivo: preferential association with the production of type 2 cytokines. Eur J Immunol 2000;30:204–10.

[12] Sallusto F, Palermo B, Lenig D, Miettinen M, Matikainen S, Julkunen I, et al. Distinct patterns and kinetics of chemokine production regulate dendritic cell function. Eur J Immunol 1999;29:1617–25.

[13] Vestergaard C, Yoneyama H, Murai M, Nakamura K, Tamaki K, Terashima Y, et al. Overexpression of Th2-specific chemokines in NC/Nga mice exhibiting atopic dermatitis-like lesions. J Clin Invest 1999;104:1097–105.

[14] Kakinuma T, Nakamura K, Wakugawa M, Mitsui H, Tada Y, Saeki H, et al. Thymus and activation-regulated chemokine in atopic dermatitis: serum thymus and activation-regulated chemokine level is closely related with disease activity. J Allergy Clin Immunol 2001;107:535–41.

[15] Godiska R, Chantry D, Raport CJ, et al. Human macrophage-derived chemokine (MDC), a novel chemoattractant for monocytes, monocyte-derived dendritic cells, and natural killer cells. J Exp Med 1997;185(9):1595–15604.

[16] Nickel R, Beck LA, Stellato C, et al. Chemokines and allergic disease. J Allergy Clin Immunol 1999;104:723–42.

[17] Imai T, Chantry D, Raport CJ, et al. Macrophage-derived chemokine is a functional ligand for the CC chemokine receptor 4. J Biol Chem 1998;273(3):1764–8.

[18] Bonecchi R, Bianchi G, Bordignon PP, et al. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J Exp Med 1998;187(1):129–34.

[19] Kakinuma T, Nakamura K, Wakugawa M, et al. Serum macrophage-derived chemokine (MDC) levels are closely related with the disease activity of atopic dermatitis. Clin Exp Immunol 2002;127(2):270–3.
[20] Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Dermatol Venereol 1980;9:44–7.
[21] Ohshima E, Otaki S, Sato H, et al. Synthesis and antiallergic activity of 11-(aminoalkylidene)-6,11-dihydrodibenz [b,e] oxepin derivatives. J Med Chem 1992;35:2074–84.
[22] Nanaka H, Otaki S, Ohshima E, et al. Unique binding pocket for KW-4679 in histamine H_1 receptor. Eur J Pharmacol 1998;345:111–7.
[23] Horikawa T, Nakayama T, Hikita I, et al. IFN-gamma-inducible expression of thymus and activation-regulated chemokine/CCL17 and macrophage-derived chemokine/CCL22 in epidermal keratinocytes and their roles in atopic dermatitis. Int Immunol 2002;14:767–73.
[24] Furukawa H, Nakamura K, Zheng X, et al. Differential effects of immunosuppressive drugs on TARC production in PBMCs of patients of atopic dermatitis. J Dermatol Sci 2004;35:35–42.
[25] Okada M, Tokumitsu H, Kubota Y, et al. Interaction of S100 proteins with the antiallergic drugs, Olopatadine, amlexanox, and cromolyn: identification of putative drug binding sites on S100A1 protein. Biochem Biophys Res Commun 2002;292:1023–30.
[26] Hamid Q, Naseer T, Minshall EM, et al. In vivo expression of IL-12 and IL-13 in atopic dermatitis. J Allergy Clin Immunol 1996;98:225–31.