CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES

Akiba H, Kehren J, Ducluzeau MT, Krasteva M, Horand F, Kaiserlian D *et al.* (2002) Skin inflammation during contact hypersensitivity is mediated by early recruitment of $CD8^+$ T cytotoxic 1 cells inducing keratinocyte apotosis. *J Immonol* 158:3079–87

Albanesi C, Scarponi C, Cavani A, Federici M, Nasorri F, Girolomoni G (2000a) Interleukin-17 is produced by both Th1 and Th2 lymphocytes, and modulates interferon-γ- and interleukin-4-induced activation of human keratinocytes. *J Invest Dermatol* 115:81–7

Albanesi C, Scarponi C, Sebastiani S, Cavani A, Federici M, De Pita O *et al.* (2000b) IL-4 enhances keratinocyte expression of CXCR3 agonistic chemokines. *J Immunol* 165:1395–402

Blazar BR, Taylor PA, Panoskaltsis-Mortari A, Vallera DA (1998) Rapamycin inhibits the generation of graft-versus-host disease- and graft-versus-leukemia-causing T cells by interfering with the production of Th1 or Th1 cytotoxic cytokines. *J Immunol* 160:5355–65

Bour H, Peyron E, Gaucherand M, Garrigue JL, Desvignes C, Kaiserlian D *et al.* (1995) Major histocompatibility complex class I-restricted $CD8^+$ T cells and class II-restricted $CD4^+$ T cells, respectively, mediated and regulate contact sensitivity to dinitrofluorobenzene. *Eur J Immunol* 25:3006–10

Dearman RJ, Kimber I (2000) Role of $CD4^+$ T helper 2-type cells in cutaneous inflammatory responses induced by fluorescein isothiocyanate. *Immunology* 101:442–51

Fujita H, Asahina A, Gao P, Fujiwara H, Tamaki K (2004) Expression and regulation of RANTES/CCL5, MIP-1α/CCL3, and MIP-1β/CCL4 in mouse Langerhans cells. *J Invest Dermatol* 122:1331–3

Fujita H, Asahina A, Sugaya M, Nakamura K, Gao P, Fujiwara H *et al.* (2005) Differential production of Th1- and Th2-type chemokines by mouse Langerhans cells and splenic dendritic cells. *J Invest Dermatol* 124:343–50

Griffiths CE, Esmann J, Fisher GJ, Voorhees JJ, Nickoloff BJ (1990) Differential modulation of keratinocyte intercellular adhesion molecule-I expression by γ interferon and phorbol ester: evidence for involvement of protein kinase C signal transduction. *Br J Dermatol* 122:333–42

He D, Wu L, Kim HK, Li H, Elmets CA, Xu H (2006) CD8+ IL-17-producing T cells are important in effector functions for the elicitation of contact hypersensitivity responses. *J Immunol* 177:6852–8

Heufler C, Koch F, Schuler G (1998) Granulocyte/macrophage colony-stimulating factor and interleukin 1 mediate the maturation of murine epidermal Langerhans cells into potent immunostimulatory dendritic cells. *J Exp Med* 167:700–5

Homey B, Alenius H, Müller A, Soto H, Bowman EP, Yuan W *et al.* (2002) CCL-CCR10 interaction regulate T cell-mediated skin inflammation. *Nat Med* 8:157–65

Kakinuma T, Nakamura K, Wakugawa M, Yano S, Saeki H, Torii H *et al.* (2002) IL-4, but not IL-13, modulates TARC (thymus and activation-regulated chemokine)/CCL17 and IP-10 (interferon-induced protein of 10 kDA)/CXCL10 release by TNF-α and IFN-γ in HaCaT cell line. *Cytokine* 20:1–6

Kaplan DH, Jenison MC, Saeland S, Shlomchik WD, Shlomchik MJ (2005) Epidermal Langerhans cell-deficient mice develop enhanced contact hypersensitivity. *Immunity* 23:611–20

Kissenpfennig A, Henri S, Dubois B, Laplace-Builhe C, Perrin P, Romani N *et al.* (2005) Dynamics and function of Langerhans cells *in vivo*: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. *Immunity* 22:643–54

Kissenpfennig A, Malissen B (2006) Langerhans cells—revisiting the paradigm using genetically engineered mice. *Trends Immunol* 27:132–9

Kitagaki H, Fujisawa S, Watanabe K, Hayakawa K, Shiohara T (1995) Immediate-type hypersensitivity response followed by a late reaction is induced by repeated epicutaneous application of contact sensitizing agents in mice. *J Invest Dermatol* 105:749–55

Kitagaki H, Ono N, Hayakawa K, Kitazawa T, Watanabe K, Shiohara T (1997) 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* 159:2484–91

Kobayashi M, Shimauchi T, Hino R, Tokura Y (2004) Roxithromycin downmodulates Th2 chemokine production by keratinocytes and chemokine receptor expression on Th2 cells: its dual inhibitory effects on the ligands and the receptors. *Cell Immunol* 228:27–33

Li J, Ireland GW, Farthing PM, Thornhill MH (1996) Epidermal and oral keratinocytes are induced to produce RANTES and IL-8 by cytokine stimulation. *J Invest Dermatol* 106:661–6

Mahalingam S, Chaudhri G, Tan CL, John A, John A, Foster PS *et al.* (2001) Transcription of the interferon γ (IFN-γ)-inducible chemokine Mig in IFN-γ-deficient mice. *J Biol Chem* 276:7568–74

Matsue H, Cruz PD Jr, Bergstresser PR, Takashima A (1992) Langerhans cells are the major source of mRNA for IL-1β and MIP-1α among unstimulated mouse epidermal cells. *J Invest Dermatol* 99:537–41

Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I *et al.* (2002) Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity* 17:375–87

Pastore S, Corinti S, La Placa M, Didona B, Girolomoni G (1998) Interferon-γ promotes exaggerated cytokine production in keratinocytes cultured from patients with atopic dermatitis. *J Allergy Clin Immunol* 101:538–44

Salgado CG, Nakamura K, Sugaya M, Tada Y, Asahina A, Fukuda S *et al.* (1999) Differential effects of cytokines and immunosuppressive drugs on CD40, B7-1, and B7-2 expression on purified epidermal Langerhans cells1. *J Invest Dermatol* 113:1021–7

Sebastiani S, Albanesi C, De PO, Puddu P, Cavani A, Girolomoni G (2002) The role of chemokines in allergic contact dermatitis. *Arch Dermatol Res* 293:552–9

Seo N, Tokura Y (1999) Downregulation of innate and acquired antitumor immunity by bystander γ/δ and α/β T lymphocytes with Th2 or Tr1 cytokine profiles. *J Interferon Cytokine Res* 19:555–61

Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B *et al.* (2002) Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 3:673–80

Stevens SR, Hanifin JM, Hamilton T, Tofte SJ, Cooper KD (1998) Long-term effectiveness and safety of recombinant human interferon γ therapy for atopic dermatitis despite unchanged serum IgE levels. *Arch Dermatol* 134:799–804

Sugita K, Kabashima K, Atarashi K, Shimauchi T, Kobayashi M, Tokura Y (2007) Innate immunity mediated by epidermal keratinocytes promotes acquired immunity involving Langerhans cells and T cells in the skin. *Clin Exp Immunol* 147:176–83

Tokura Y, Yagi J, O'Malley M, Lewis JM, Takigawa M, Edelson RL *et al.* (1994) Superantigenic staphylococcal exotoxins induce T-cell proliferation in the presence of Langerhans cells or class II-bearing keratinocytes and stimulate keratinocytes to produce T-cell-activating cytokines. *J Invest Dermatol* 102:31–8

Tsuda T, Tohyama M, Yamasaki K, Shirakata Y, Yahata Y, Tokumaru S *et al.* (2003) Lack of evidence for TARC/CCL17 production by normal human keratinocytes *in vitro*. *J Dermatol Sci* 31:37–42

Wolf SF, Sieburth D, Sypek J (1994) Interleukin 12: a key modulator of immune function. *Stem Cells* 12:154–68

Xiao T, Fujita H, Saeki H, Mitsui H, Sugaya M, Tada Y *et al.* (2003) Thymus and activation-regulated chemokine (TARC/CCL17) produced by mouse epidermal Langerhans cells is upregulated by TNF-α and IL-4 and downregulated by IFN-γ. *Cytokine* 23:126–32

Xu H, Dilulio NA, Fairchild RL (1996) T cell populations primed by hapten sensitization in contact sensitivity are distinguished by polarized patterns of cytokine production: interferon γ-producing (Tc1) effector $CD8^+$ T cells and interleukin (IL) 4/IL-10-producing (Th2) negative regulatory $CD4^+$ T cells. *J Exp Med* 183:1001–12

Ying S, Kikuchi Y, Meng Q, Kay AB, Kaplan AP (2002) TH1/TH2 cytokines and inflammatory cells in skin biopsy specimens from patients with chronic idiopathic urticaria: comparison with the allergen-induced late-phase cutaneous reaction. *J Allergy Clin Immunol* 109:694–700

Papuloerythroderma of Ofuji induced by furosemide

To the Editor: Papuloerythroderma was first described in 1984 by Ofuji et al.[1] It may occur as a result of allergic reaction to unknown substances with T helper (Th) 2 deviation.[2,3] Recently, we have found that one of the causative agents of papuloerythroderma is a drug, as documented in a patient administered with aspirin.[4] Here, we describe a case of papuloerythroderma caused by furosemide.

A 79-year-old man had a 25-year history of mild eczema, which was originally prominent on his trunk and spread thereafter to his 4 extremities. He also had experienced heart failure 10 months earlier and was given furosemide 6 months before his visit to our clinic. Four months after the administration, an erythematous papular eruption deteriorated and he was referred to our hospital.

Clinical examination revealed widespread eythroderma with coalescent solid papules predominantly on the trunk and extremities with sparing of the face, axillae, and skinfolds (Fig 1, *A*). The peripheral blood sample showed a normal leukocyte count of 5100/μL with 13.3% eosinophils (678/μL). A biopsy specimen showed moderate perivascular infiltration of lymphocytes and eosinophils in the papillary dermis. Although a patch test to furosemide produced negative findings, the lymphocyte stimulation test produced positive findings (stimulation index 9.08; normal < 1.8) (Fig 1, *B*).

Furosemide therapy was discontinued and he was treated with topical application of 0.05% betamethasone butyrate propionate once a day for a month, resulting in clinical improvement. An oral challenge test to furosemide revealed positive results, as the patient developed the same erythematous papular eruption 48 hours after administration. Upon this provocation, a higher percentage of $CCR4^+$ $CD4^+$ Th2 cells was found in the peripheral blood (Fig 1, *C*), and $HLA\text{-}DR^+CD4^+$ activated T cells were elevated to 15.2%. Therefore, we gave this patient the diagnosis of papuloerythroderma caused by furosemide, and the eruption seemed to be mediated by Th2 cells reactive with furosemide.

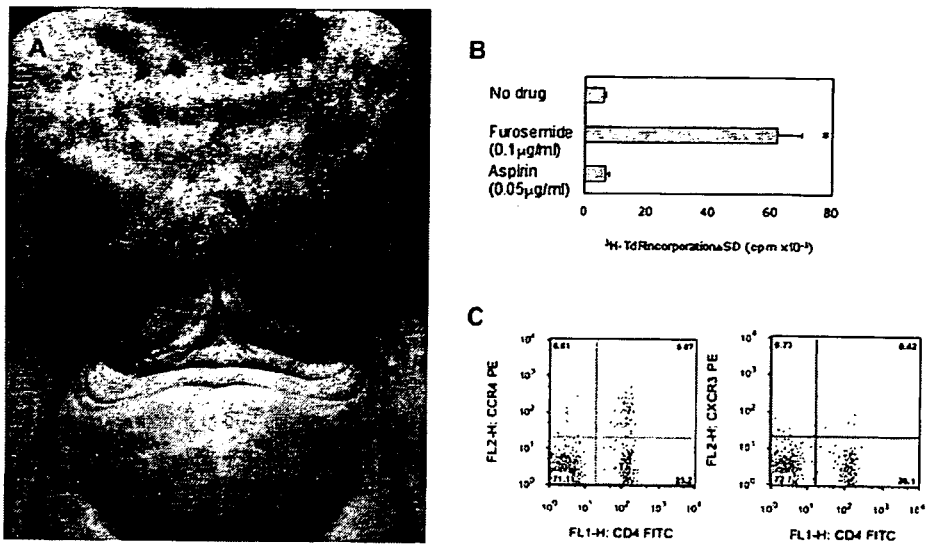

Fig 1. **A**, Diffuse coalesced red papules form sheets of erythroderma-like lesions on trunk with sparing of skinfolds. **B**, Lymphocyte stimulation test. Patient's peripheral blood mononuclear cells (PBMC) were cultured for 72 hours with furosemide or aspirin as control. ^{3}H-thymidine was pulsed for last 18 hours. $^*P < .01$, compared with control. **C**, Flow cytometric analysis of patient's PBMC after provocation test. *FITC*, fluorescein isothiocyanate; *PE*, phycoerythrin.

We have recently reported a patient with aspirin-induced papuloerythroderma.[4] The patient described here is the second case that was evoked by a drug. In both cases, the eruptions were different from the acute type of exanthema usually observed as drug eruption. Rather, they were chronically seen presumably in association with pre-existing eczematous dermatitis. It should be noted that widely used drugs are one of the important causative agents for papuloerythroderma.

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REFERENCES

1. Ofuji S, Furukawa F, Miyachi Y, Ohno S. Papuloerythroderma. *Dermatologica* 1984;169:125-30.
2. Wakeel RA, Keefe M, Chapman RS. Papuloerythroderma: another case of a new disease. Arch Dermatol 1991;127:96-8.
3. Hasegawa W, Tachibana T, Ito K, Ito M, Toma H, Sato Y. Papuloerythroderma of Ofuji associated with strongyloidiasis. J Dermatol 2003;30:157-8.
4. Sugita K, Koga C, Yoshiki R, Izu K, Tokura Y. Papuloerythroderma caused by aspirin. Arch Dermatol 2006;142:792-3.

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LETTER TO THE EDITOR

Fexofenadine downmodulates antigen-presenting ability of murine epidermal Langerhans cells

KEYWORDS
Langerhans cell; Dendrtic cell; Fexofenadine; T cell

To the Editor

Fexofenadine is a nonsedating, anti-allergic, histamine H1-receptor-blocking antagonist acknowledged as an oral drug for allergic rhinitis, urticaria, and various itchy skin diseases including atopic dermatitis and other eczematous dermatoses [1]. This H1 blocker suppresses the production of various chemical mediators as anti-allergic action. Furthermore, fexofenadine inhibits the production of cytokines and chemokines by epidermal keratinocytes and nasal epithelial cells, such as CCL17/TARC, and CCL22/MDC [2–4]. It also inhibits the production of CCL5/RANTES in keratinocytes [4]. Therefore, fexofenadine downregulates the production of Th2 chemokines and eosinophil chemokine CCL5 by keratinocytes, thereby possibly preventing skin-infiltration of Th2 cells and eosinophils. Susceptibility of keratinocytes to fexofenadine was also found in downregulated expression of CD54/ICAM-1 by this anti-histamic drug [4].

In cutaneous immunity, Langerhans cells (LCs) are professional antigen-presenting cells in the epidermis and play a critical role in the development of contact hypersensitivity with the help of keratinocytes. Since fexofenadine suppresses the early and late cutaneous allergic responses [5], it is possible that fexofenadine modulates the immunological functions of not only keratinocytes but also LCs. We therefore investigated the effect of fexofenadine on the hapten-presenting capacity of LCs.

First, epidermal cell (EC) suspensions freshly isolated from naïve BALB/c mice were subjected to Ficoll gradient separation of LC-enriched epidermal cells (LC-ECs) as described previously [6]. The percentage of LCs 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). LC-ECs were modified with trinitrophenyl (TNP) [6]. As responders, $CD4^+$ T cells were prepared from lymph node cells (LNCs) of trinitrochlorobenzene (TNCB)-sensitized BALB/c mice. The immune $CD4^+$ T cells were cultured with TNP-haptenized LC-ECs to examine T cell

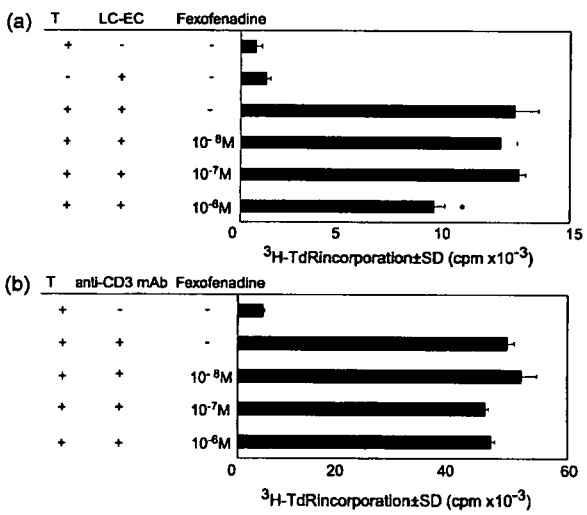

Fig. 1 Fexofenadine suppression of the ability of LC-ECs to present hapten to immune T cells without direct suppression of T cells. (a) LC-ECs were obtained from naïve BALB/c mice and modified with trinitrophenyl (TNP). Immune LNCs were obtained from BALB/c mice sensitized with 2,4,6-trinitrochlorobenzene (TNCB) 5 days before. $CD4^+$ T cells were isolated positively with monoclonal anti-CD4 antibody using MACS, and their purity was >98%. Immune lymph node $CD4^+$ T cells (2×10^5 per well) were cultured for 72 h with TNP-modified LC-ECs (5×10^3 per well) in the presence or absence of fexofenadine and pulsed with methyl [^{3}H] thymidine (1 μCi/well) 18 h before harvest. Cells were collected on glass fiber filters using a cell harvester and their radio-uptake was measured in a scintillation counter. Data represent the mean ± S.D. $^*P < 0.05$, compared with T + LC-EC group. (b) Immune $CD4^+$ T cells (1×10^6 per well) were cultured for 72 h with varying concentrations of fexofenadine in the presence of anti-CD3 mAb (5 μg/ml).

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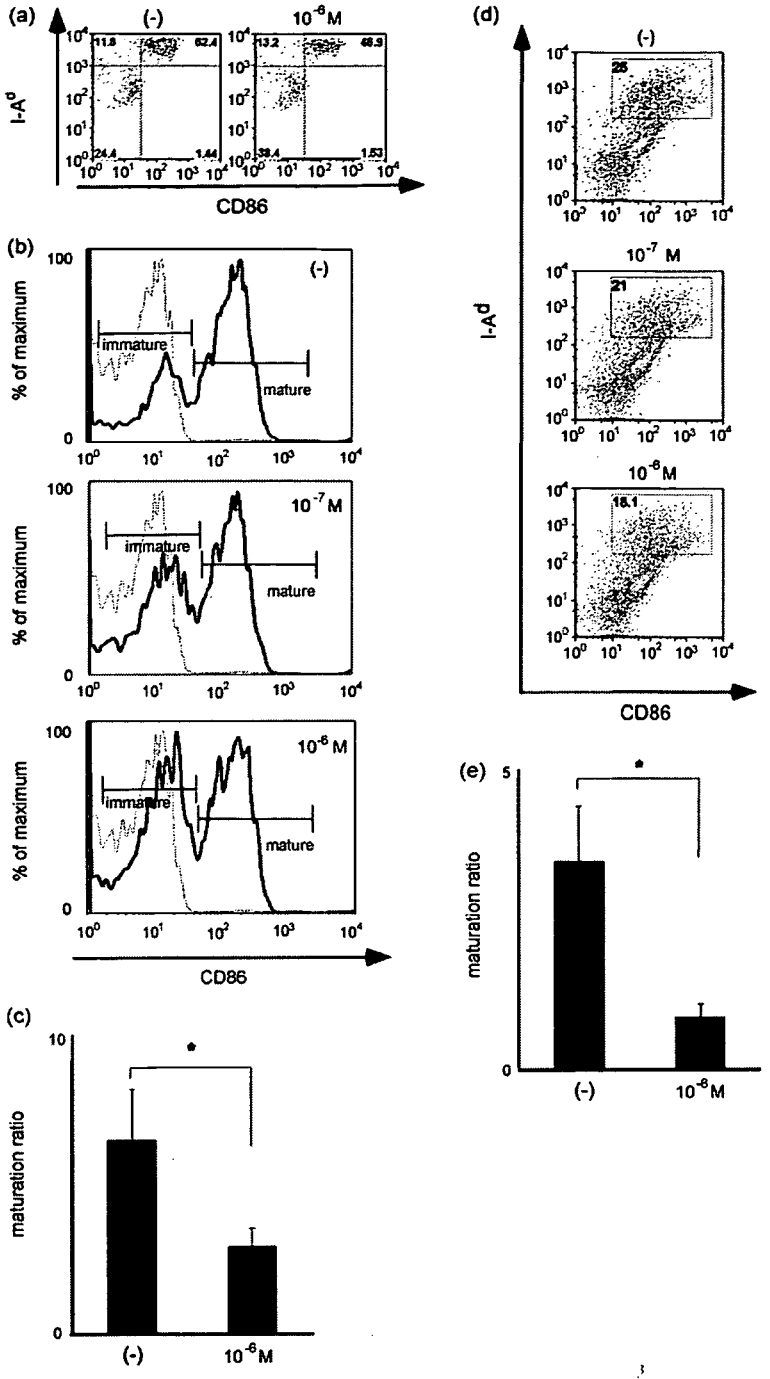

Fig. 2 Fexofenadine downmodulation of CD86 expression on LCs. (a) Epidermal cell suspensions from naïve mice were cultured for 24 h with or without fexofenadine at 10^{-6} M. The cultured cells were subjected to flow cytometric analysis to see the expression of CD86 on LCs, which were gated by I-A^d expression. Fexofenadine did not impair the viability of ECs at the concentrations used. (b) A representative flow cytometry shows the mature (CD86 high) and immature (CD86 intermediate) populations of LCs in the culture system. The dotted line represent the isotype-matched control. (c) The mature/immature ratio is calculated from three independent experiments. Data represent the mean ± S.D. $^*P < 0.05$. (d) Fexofenadine reduction of the percentage of $CD86^+$ BMDC population. Murine immature DCs were generated from bone marrow according to standard protocols [8,9]. Minor modification included feed culture medium on day 3 containing GM-CSF (10 ng/ml). On day 6, BMDCs (5×10^6 per well) were cultured for 24 h with the two indicated concentrations of fexofenadine and the expression of CD86 on BMDCs, which were gated by I-A^d, was monitored. Three independent series of experiments confirmed this reduction of mature BMDC number by fexofenadine. Fexofenadine did not impair the viability of BMDCs at the concentration used. (e) The mature/immature ratio is calculated from three independent experiments. Data represent the mean ± S.D. $^*P < 0.01$.

proliferation. The culture was maintained for 72 h in the presence or absence of fexofenadine at 10^{-6} to 10^{-8} M.

As shown in Fig. 1a, the ability of LC-ECs treated with 10^{-6} M fexofenadine to stimulate immune T cells was significantly lower than that of LC-ECs incubated without fexofenadine. A question arose whether this reduction stemmed from fexofenadine impairment of LCs or T cells. In an alternative system, precultivation of LCs with fexofenadine before coculturing with T cells decreased the response (data not shown). These data strongly suggest that fexofenadine suppresses LC function. Moreover, when purified $CD4^+$ T cells from trinitrochlorobenzene-sensitized mice were solely cultured for 72 h with anti-CD3 mAb in the presence of varying concentrations of fexofenadine, the stimulant-induced proliferation of T cells was not affected by fexofenadine at 10^{-6} M (Fig. 1b). Thus, fexofenadine did not directly decrease T cell proliferation, and its suppressive effect on LCs seemed to be predominant.

B7 molecules such as CD86 are involved in the antigen-presenting ability of LCs. To test the effect of fexofenadine on LC CD86 expression, freshly isolated ECs were cultured for 24 h in the presence or absence of fexofenadine and double-stained with phycoerythrin (PE)-labeled anti-I-A^d and fluorescein isothiocyanate (FITC)-labeled anti-CD86 monoclonal antibodies (BD PharMingen, San Diego, CA) and were gated by I-A^d expression. As shown in Fig. 2a, the percentage of $CD86^+$I-A^{d+} LCs were decreased by fexofenadine treatment. LCs usually have two populations in their CD86 expression after 24 h culture [6]. Fexofenadine decreased the CD86-highly expressing population (mature) of LCs, as the immature population was increased by fexofenadine at 10^{-7} or 10^{-6} M (Fig. 2b). Three independent series of experiments confirmed the reduction of mature LCs by fexofenadine (Fig. 2c).

In our experiment systems, keratinocytes coexist with LCs. This raises the possibility that fexofenadine alters the production of cytokines by bystander keratinocytes, thereby modulating the function of LCs. Moreover, recent findings have suggested that dermal dendritic cells (DCs) positively induce contact hypersensitivity, while LCs are rather regulatory [7]. Therefore, another DC population should be tested without contamination of keratinocytes. For this purpose, bone marrow-derived dendritic cells (BMDCs) were cultured for 24 h with fexofenadine and the I-A^d- and CD86-double positive cells was monitored by flow cytometry. As shown in Fig. 2d, fexofenadine decreased the percentage of $CD86^+$ mature BMDCs. Three independent series of experiments confirmed this reduction of mature BMDCs (Fig. 2e). The above findings interpreted as an indication that fexofenadine downregulates the costimulatory molecules of LCs, and subsequently inhibits their antigen-presenting function. To address this possibility, BMDCs derived from C57/BL6 mice were harvested on day 6 and treated with 25 μg/ml mitomycin C (Sigma) for 30 min at 37 °C. The treated BMDCs (2×10^5 per well) were then cultured with $CD4^+$ T cells (2×10^5 per well) derived from BALB/c mice in 96-well cell culture plates for 3 days. The cells were pulsed with methyl [^{3}H] thymidine. T cell proliferation was markedly inhibited at fexofenadine 10^{-7} M (data not shown). Therefore, it is likely that fexofenadine directly suppresses the antigen-presenting function of DCs though downmodulation of the costimulatory molecules.

In our study, we showed that fexofenadine suppresses the hapten-presenting ability of LCs. This suppression was associated at least partly with the decreased expression of costimulartory molecules. Our study implicates that fexofenadine downmodulates DC function concerned with skin immunity and may exerts its therapeutic effectiveness.

Conflict of interest

The authors state that this study was financially supported partly by Sanofi-Aventis.

References

[1] Kawashima M, Tango T, Noguchi T, Inagi M, Nakagawa H, Harada S. Addition of fexofenadine to a topical corticosteroid reduces the pruritus associated with atopic dermatitis in a 1-week randomized, multicentre, double-blind, placebo-controlled, parallel-group study. Br J Dermatol 2003;148: 1212–21.

[2] Gelfand EW, Cui ZH, Takeda K, Kanehiro A, Joetham A. Fexofenadine modulates T-cell function, preventing allergen-induced airway inflammation and hyperresponsiveness. J Allergy Clin Immunol 2002;110:85–95.

[3] Gelfand EW, Cui ZH, Takeda K, Kanehiro A, Joetham A. Effects of fexofenadine on T-cell function in a murine model of allergen-induced airway inflammation and hyperresponsiveness. J Allergy Clin Immunol 2003;112:S89–95.

[4] Kobayashi M, Tokura Y. Preferential downmodulation of certain chemokines by fexofenadine in human keratinocytes. J Dermatol Sci 2005;38:67–9.

[5] Simons FE, Johnston L, Gu X, Simons KJ. Suppression of the early and late cutaneous allergic responses using fexofenadine and montelukast. Ann Allergy Asthma Immunol 2001;86: 44–50.

[6] Sugita K, Kabashima K, Atarashi K, Shimauch 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;147:176–83.
[7] Kissenpfennig A, Henri S, Dubois B, Laplace-Builhe C, Perrin P, Romani N, et al. Dynamics and function of Langerhans cells in vivo: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. Immunity 2005;22:643–54.
[8] Inaba K, Inaba M, Romani N, Aya H, Deguchi M, Ikehara S, 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;176:1693–702.
[9] Vabulas RM, Braedel S, Hilf N, Singh-Jasuja H, Herter S, Ahmad-Nejad P, 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;277:20847–53.

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Review Article

Epidermal chemokines and modulation by antihistamines, antibiotics and antifungals

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Abstract: Growing evidence has demonstrated that chemokines released from epidermal cells control inflammatory skin diseases. Keratinocytes elaborate both Th1- and Th2-associated chemokines, although the former is more abundantly produced than the latter. Downmodulation of keratinocyte production of chemokines is one of the therapeutic approaches for cutaneous inflammatory disorders. Recent observations have shown that keratinocyte chemokine production can be modulated by well-used drugs, including antihistamines, antibiotics and antifungals. Utilization of the beneficial side effects of these drugs may by clinically valuable.

Key words: antibiotic – antifungal – antihistamine – chemokine – epidermis

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Introduction

Skin is a well-orchestrated immune organ where epidermal cells produce various cytokines, chemokines and mediators, thereby inducing leucocytes infiltration in the dermis and even epidermis and evoking contact dermatitis and other skin lesions (1). Recent accumulating evidence has clarified that chemokines play an important role in cutaneous immunity (2). Allergic contact dermatitis and atopic dermatitis (AD) are the typical conditions to show the essential roles of chemokines by which immunocompetent cells interact dynamically with each other (3,4). Keratinocytes are the major producers of epidermal chemokines, which are controlled by various cytokines (5–7).

Keratinocyte production of chemokines may be modulated by clinically used therapeutic reagents. Cytokines such as interferon-γ (IFN-γ) and anticytokine antibodies such as antitumor necrosis factor-α (TNF-α) duly alter the chemokine production as seen in patients with mycosis fungoides treated with IFN-γ (8) and psoriasis with anti-TNF-α antibody (9). Besides these biologics or biological response modifiers, recent findings have revealed that certain drugs have a capacity to modulate the chemokine production. These seemingly attractive drugs include antihistamines, antibiotics and antifungals. The fact that antihistamines have this capacity is perhaps not particularly surprising, as it has been known for many years that antihistamines alter cytokine production. However, it is of particular interest that antibiotic and antifungal drugs have this potential. We can hypothesize that they possibly exert beneficial, not adverse, side effects in patients with not only skin allergy but also bacterial/fungal infections, although physicians and patients have not noticed such pleasant effects of the drugs. This article aims to review the roles of epidermal chemokines and highlight the drugs capable of modulating keratinocyte chemokine production.

Epidermal chemokines

In the skin, external stimuli such as chemicals and ultraviolet B (UVB) (10), or cytokines represented by IFN-γ and TNF-α (6,11) stimulate epidermal keratinocytes to elaborate various chemokines, which initiate migration of T cells as well as polymorphonuclear leucocytes (12). The infiltrating T cells further activate keratinocytes to produce chemokines by secreting IFN-γ (13), leading to exaggeration of cutaneous inflammation.

Chemokines in the epidermis are mainly released by keratinocytes and Langerhans cells (LC). These two types of cells are deeply involved in skin immunity, as contact dermatitis is induced and elicited by their close interaction (14). Table 1 summarizes chemokines produced/expressed by keratinocytes (15–18) and LC (19–22), and Table 2 shows chemokine receptors expressed on Th1, Th2 and LC. Keratinocytes are capable of producing Th1-associated chemokines (Th1 chemokines) with affinity to

Table 1. Chemokines produced by keratinocytes and LCs

Cell sources	Chemokines	Receptors for chemokines	Chemoattracted cells
Keratinocytes	Mig/CXCL9	CXCR3	Th1 cells
	IP-10/CXCL10	CXCR3	Th1 cells
	I-TAC/CXCL11	CXCR3	Th1 cells
	MDC/CCL22	CCR4	Th2 cells
	(TARC/CCL17)	(CCR4)	(Th2 cells)
	RANTES/CCL5	CCR1, CCR3, CCR5	Eosinophils, T cells, fibroblasts
	CTACK/CCL27	CCR10	Skin homing memory T cells
	LARC/MIP-3α/CCL20	CCR6	Memory T cells, monocytes, immature DCs, LCs
	MCP-1/CCL2	CCR2	Monocytes, DC precursors
	IL-8/CXCL8	CXCR2	Neutrophils
	GROα/MGSA	CXCR2	Neutrophils
	I-309/CCL1	CCR8	Th2 cells, LC precursors
LCs	TARC/CCL17	CCR4	Th2 cells
	MDC/CCL22	CCR4	Th2 cells
	RANTES/CCL5	CCR1, CCR3, CCR5	Eosinophils, T cells, fibroblasts
	MIP-1α/CCL3	CCR1, CCR5	Monocytes, T cells
	MIP-1β/CCL4	CCR5	Th1 cells

Keratincyte-derived chemokines were investigated by ELISA and/or RT-PCR of *in vitro* cultured normal human epidermal keratinocytes and their supernatants, or by immunohistochemistry and/or *in situ* hybridization of skin specimens (9,15–18). It should be noted that CCL17 can not be produced by normal human keratinocytes (25), although it is released from HaCaT cells. CCL1 was detected *in vitro*, but there has been no report on the *in vivo* expression of CCL1. LC-derived chemokines were examined by RT-PCR or ELISA of purified murine LCs and their supernatants (19–21), or by and human LC histiocytosis tumor cells (22).

Table 2. Chemokine receptors in Th1, Th2, and LCs

Cell type	Chemokine receptors	Ligands (chemokines)
Th1 (Tc1)	CXCR3	IP-10/CXCL10
		Mig/CXCL9
		I-TAC/CXCL11
	CCR5	RANTES/CCL5
		MIP-1α/CCL3
		MIP-1β/CCL4
Th2	CCR4	TARC/CCL17
		MDC/CCL22
	CCR3	eotaxin-1/CCL11
		Eotaxin-2/CCL24
		RANTES/CCL5
		MCP-3/CCL7
		MCP-4/CCL14
	CCR8	I-309/CCL1
LCs	CCR6	MIP-3α/CCL20 (epidermis)
	CCR7	SLC/CCL21 (lymph nodes)
	CXCR4	SDF-1/CXCL 12 (lymphatics, lymph nodes)

Chemokine receptors expressed on Th1 and Th2 cells (23) and LCs (45) were summarized.

CXC chemokine receptor 3 (CXCR3) and CC chemokine receptor 5 (CCR5) on Th1 cells (23), including IFN-γ-inducible protein 10 (IP-10/CXCL10), monokine induced by IFN-γ (Mig/CXCL9) and IFN-γ-inducible T-cell chemoattractant (I-TAC/CXCL11) and Th2-associated chemokines (Th2 chemokines) with affinity to CCR4, CCR3 and CCR8 on Th2 cells (23), including thymus and activation-regulated chemokine (TARC/CCL17) and macrophage-derived chemokine (MDC/CCL22) (2,9,15–18). It should be carefully noted that CCL17 is produced by the keratinocyte cell line HaCaT cells but not cultured normal human keratinocytes (24,25). In addition, RANTES/CCL5, chemotactic for eosinophils and Th1 and Th2 cells, and interleukin (IL)-8 for neutrophils are important chemokines produced by keratinocytes in consideration of skin immune or inflammatory disorders. Thus, a considerably wide range of chemokines is released from the keratinocytes.

Furthermore, a specialized form of chemokine/receptor system is constructed by the interaction between CTACK/CCL27 secreted by keratinocytes and CCR10 on certain population of T cells with skin-homing capacity (26,27). T cells bearing cutaneous lymphocyte-associated antigen (CLA) is known as skin-homing T cells. CCR4 and CCR10 show preferential expression on circulating CLA^+

skin-homing T cells. While the majority of circulating $CD4^+$ CLA^+ memory T cells expresses CCR4, 30–40% of CLA^+ memory T cells are positive for CCR10 (28). As CCL27 expression is not concomitant with other chemokines such as CXCL10 in hapten-challenged skin (26), $CCR10^+$ T cells possibly play a different role from $CXCR3^+$ T cells in the cutaneous sensitivity.

Several studies by different groups of investigators have suggested that the chemokine production patterns are different between keratinocytes and LC. Although keratinocytes strongly produce Th1 chemokines, LC markedly express Th2 chemokines CCL17 and CCL22 (19–22). Accordingly, when epidermal cells are deprived of LC, the expression of CCL17 and CCL22 are markedly reduced. LC production of Th2 chemokines is inhibited by IFN-γ (27) and enhanced in IFN-γ-deficient mice.

Allergic contact dermatitis as a representative system where epidermal chemokines are involved

Murine contact hypersensitivity (CHS), corresponding clinically to allergic contact dermatitis, is elicited as a consequence of immunological reactions induced by skin application of antigen, cellular interactions among LC, T cells, keratinocytes and mast cells, and well-organized participation of a variety of cytokines/chemokines (29). Antigen-presenting LC or dermal dendritic cells (DC) are prime T cells in the induction or afferent limb of the CHS and restimulate them in the elicitation or efferent limb, and chemokines and their receptors are involved in these dynamic responses (1,2,29) (Fig. 1). Recent findings have rendered the positive and regulatory roles of LC and dermal DC mysterious, and in particular, LC have been suggested to play a suppressive role (30,31).

Historically, the expression of chemokine genes in CHS responses has been studied along with the discovery of new chemokines of keratinocyte origin (32,33). In murine CHS to picryl chloride, mRNA for CXCL10 as well as macrophage chemoattractant protein 1 (MCP-1/CCL2) is expressed as early as 4 h after challenge and remains elevated until 24 h (34). CXCL10 is also expressed in CHS to dinitrofluorobenzene and oxazolone (35). Although the sources of CXCL10 at the elicited skin sites had been controversial (34–36), human keratinocytes were documented to produce CXCL10 and CXCL9 upon stimulation with IFN-γ (7,36–38). Furthermore, CXCL10 is most abundantly and predominantly expressed on epidermal cells at patch test sites of human allergic contact dermatitis (36). In their study, CXCL9 is expressed in both epidermis and dermis.

It is proposed that the expression of CXCR3-agonistic chemokines by epidermal and dermal cells contribute to an environment in which activated T cells bearing CXCR3 migrate to the site of allergen reaction, thereby enhancing

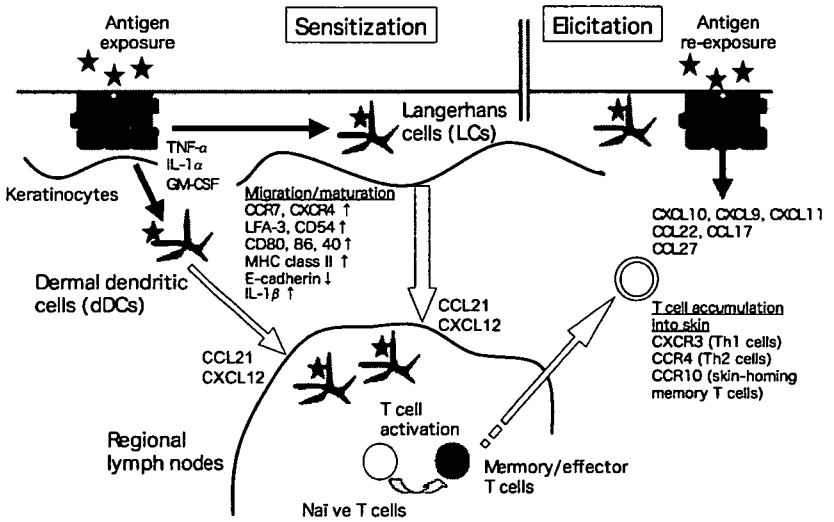

Figure 1. Schematic representation of mechanisms of contact hypersensitivity (CHS) and participation of keratinocyte chemokines. Sensitization phase of CHS: upon exposure to exogenous antigens, epidermal keratinocytes produce tumor necrosis factor (TNF)-α, interleukin (IL)-1α and GM-CSF, thereby inducing the migration and maturation of cutaneous dendritic cells (DC) [epidermal Langerhans cells (LC) and dermal DC]. LC with high expression of CCR7 and CXCR4 migrate to the regional lymph nodes where their ligands, CCL21/SLC and CXC12/SDF-1, are released. The migrated and antigen-bearing cutaneous DC sensitize the T cells. Elicitation phase of CHS: upon re-exposure to the antigen, keratinocytes produce Th1 chemokines (CXCL10/IP-10, CXCL9/Mig and CXCL11/I-TACK), Th2 chemokines (CCL22/MDC and possibly CCL17/TARC) and skin-homing memory T-cell chemokine (CCL27/CTACK). Ordinary haptens stimulate keratinocytes to produce Th1 chemokines, and the migrated Th1/Tc1 cells further induce keratinocyte Th1 chemokine production by releasing interferon-γ. LC are an important source of Th2 chemokines, but it remains unclear whether LC-derived Th2 chemokines attract Th2 cells in some *in vivo* settings.

and maintaining the inflammatory infiltrate. In this scenario, $CD8^+$ T cells are suggested to play a major role for induction of CXCL10 via secreted IFN-γ. In fact, T cells bearing CXCR3 preferentially infiltrate at the challenge sites compared with the T cells bearing CCR4 (13). CXCL10 produced locally at the challenge sites induces infiltration of $CXCR3^+$ T cells, and their $CD8^+$ T cell population further stimulates the local milieu to produce CXCL10 by IFN-γ, leading to an enhancement of the elicited responses (13).

The expression of CXCL10 and CXCL9 is followed by that of CCL27 (26), suggesting that different population of T cells with CCR10 migrate to the challenged skin after Th1/Tc1 infiltration. The chemotactic activity seems to be different between $CD4^+$ and $CD8^+$ T cells in response to CCL27 (27). It is an interesting issue whether the $CD4^+$ population plays a positive or regulatory role in CHS. Whereas $CD4^+$ T cells were shown to enhance $CD8^+$ effector T cell activity (39), another study has suggested that $CD4^+$ T cells serve as regulatory T cells that suppress CHS response (40,41).

In photoallergic contact dermatitis to ketoprofen, however, lymph node cells from photosensitized mice express high levels of mRNA for Th2 cytokine (IL-4) and Th2 chemokine receptor (CCR4) as well as Th1 cytokine (IFN-γ) and Th1 chemokine receptor (CXCR3) (42). Moreover, epidermal cells from challenged ear lobes have increased levels of both Th1 (CXCL9) and Th2 (CCL17) chemokines and cytokines (43,44). Therefore, it is considered that not only Th1 but also Th2 cells participate in the pathogenesis of photoallergic contact dermatitis, suggesting that there are differences in chemokine expression between the cutaneous hypersensitivities.

Chemokines produced by LC include CCL17, CCL22, CCL5, CCL3 and CCL4 (20,21). Although exogenous stimulus is mandatory for the production of substantial amounts of Th1 chemokines, CXCL10, CXCL9 and CXCL11, both in LC and splenic DC, LC exhibit low ability to produce Th1 chemokines in comparison with splenic DC. As for the Th2 chemokines, LC, but not splenic DC, produce high levels of CCL22 and CCL17 constitutively during culture even without exogenous stimuli. The production of Th2 chemokines is regulated in a complicated manner. In particular, IL-4 upregulates and IFN-γ downregulates both CCL22 and CCL17 production by LC. Of note, LC produce more amounts of Th2 chemokines than splenic DC under any conditions tested (21). The fact that LC can secrete such Th2 chemokines and eosinophil-attracting CCL5 appears to be in accordance with the recent finding that LC function as suppressors rather than as positive antigen-presenting cells (30,31). Given that dermal DC play a positive role for CHS, it is an interesting issue whether they have a pattern of chemokine production different from LC. As for the chemokine receptor expression, LC are known to bear CCR7 for migration to regional lymph nodes, but our recent study has shown that LC also express CXCR4 as a functional molecule for migration to the lymph nodes (45).

Antihistamine drugs as a modulator of keratinocyte chemokine production

The second generation of histamine H1-receptor-blocking antagonists is used for various inflammatory skin disorders. Patients with urticaria are usually well treated with antihistamines. Its antipruritic potential in the management of AD is statistically significant (46) but may be limited in such an eczematous dermatitis. In addition to antihistaminic action, they have various antiallergic actions, as represented by mast cell stabilization and resultant suppression of various chemical mediators and cytokines, such as leukotrienes, arachidonic acid, IL-6, IL-8 and TNF-α (44). Some of the antihistamines also suppress the expression of co-stimulatory molecules (47), eosinophil chemotaxis (48), adhesion molecule expression (48–51) and substance P release (51). Although they have similarities in their antihistaminic and antiallergic actions, each of them has different characteristic effects on immunocompetent cells and allergy-associated molecules.

In addition to these actions, the suppression of keratinocyte chemokine production has been demonstrated in several antihistamines (50,52,53). In a series of our study, we stimulated normal human epidermal keratinocytes or HaCaT cells by the previously reported method using IFN-γ and TNF-α as follows: 2000 units/ml of IFN-γ and 4000 units/ml of TNF-α for the first 2 h, followed by 200 units/ml of IFN-γ and 400 units/ml of TNF-α for the remaining 3 days (52,53) in the presence or absence of antihistamines. The concentration of each chemokine was measured by enzyme-linked immunosorbent assay (ELISA) and mRNA expression was evaluated by reverse transcriptase-polymerase chain reaction (RT-PCR). HaCaT cells were used to see the effect on CCL17 production, because normal human keratinocytes, at least when cultured *in vitro*, are unable to produce CCL17. By flow cytometry, we also monitored the changes in the expression of major histocompatibility complex (MHC) class II and CD54 molecules on HaCaT cells after culturing with antihistamines (52,53). The results are summarized in Table 3. There are discernible differences among antihistamines in the downmodulatory effects on each chemokine production.

Epinastine inhibits the production of Th1 chemokines, CXCL10, CXCL9 and CXCL11 (52). The optimally inhibitory dose of epinastine was 10^{-8} M, and its concentration in the skin was reported to be 10^{-7} M, suggesting that epinastine exerts its clinical effectiveness in the skin. CCL22,

Table 3. Downmodulatory effects of the three categories of drugs on keratinocytes and LCs

Target cells	Downmodulation	Antihistamines	Antibiotics	Antifungals
Keratinocytes	Proinflammatory cytokines	Olopatadine (56), cetirizine (a), levocetiridine (a), loratadine (c), bepotastine (b)	Roxithromycin (70), nadifloxacin (76)	
	CXCL8/IL-8	Cetirizine (a), levocetirizine (a)		Liranaftate (d)
	Th1 chemokines	epinastine (52), levocetirizine (a), bepotastine (b), olopatadine (56)		
	Th2 chemokines	Fexofenadine (53), bepotastine (b), loratadine (c)	roxithromycin (11,75)	
	CCL5/RANTES	Epinastine (52), fexofenadine (53)		Ketoconazole (84), terbinafine (84)
	CCL27/CTACK			Ketoconazole (84), terbinafine (84)
	MHC class II expression	Epinastine (52), cetirizine (a), levocetirizine (a)	Roxithromycin (70), nadifloxacin (77)	
	CD54 expression	Epinastine (52), cetirizine (a), levocetirizine (a), bepotastine (b)		
	Superantigen presentation		Roxithromycin (70), nadifloxacin (77)	
LCs	MHC class II expression	olopatadine (56)	Roxithromycin (68), nadifloxacin (77)	
	Co-stimulatory molecule expression		Roxithromycin (68)	
	Proinflammatory cytokines		Roxithromycin (68)	

The numbers in parentheses indicate the References.
[a,b]The data were shown as abstracts in Annual Meeting of Japanese Society of Allergology: [a]Kobayashi M, Tokura Y. Effects of levocentiridine on normal human epidermal keratinocytes: comparison with cetiridine. Jpn J Allergology 54: 1112: 2005 (abstract). [b]Kobayashi M, Kabashima K, Tokura Y. Effects of bepotastine on production of cytokines/chemokines and expression of ICAM-1 in normal human epidermal keratinocytes. Jap J Allergology 56: 347, 2007 (abstract).
[c]Unpublished observation (Kobayashi M, Tokura Y).
[d]Kobayashi M, Tokura Y. Enhancement of keratinocyte IL-8 production by β-glucan and its suppression by liranaftate. Jpn J of Med Mycol (abstract).

a Th2 chemokine, and CCL5, capable of chemoattracting Th1 and Th2 cells and eosinophils, tend to be suppressed by this antihistamine. Thus, epinastine is characterized by its wide range of suppressive capacity towards Th1 and Th2 chemokines with the former being more suppressed.

Fexofenadine suppresses Th2 chemokines, CCL22 and CCL17 and additionally CXCL11 and CCL5 as low as 10^{-7} to 10^{-5} M in a dose-dependent manner (53). As the C_{max} of fexofenadine is 1.0×10^{-6} M when administered orally at 120 mg in humans and its concentration in the skin is higher than that in the plasma, this *in vitro* suppressive concentration is considered to be meaningful. Neither production of CXCL10, CXCL9 nor CXCL8 is affected by fexofenadine. Thus, fexofenadine uniquely downregulates the production of Th2 chemokines, CCL22 and CCL17, but not Th1 chemokines, CXCL10 or CXCL9, which suggests its beneficial effects on Th2 cell-mediated, and thus, allergic cutaneous disorders. It is interesting that this Th2-preponderant suppression of chemokine production is virtually the same as UVB (10). In addition, as fexofenadine inhibits the production of CCL5, but not CXCL8, this antiallergic seems to prevent the skin infiltration of eosinophils but not neutrophils. Given that the late-phase cutaneous reaction is mediated by Th2 cells and eosinophils, fexofenadine may be effective especially for oedematous and erythematous lesions of AD. In this context, it should be carefully noted that the chronic eczematous lesion of AD is induced by Th1 cells.

Cetirizine inhibits the release of CCL2 and CCL5 from IFN-γ-stimulated keratinocytes (54). It also suppresses the production of CXCL8 as well as the expression of MHC class II and CD54 molecules in our preliminary study. Levocetiridine is virtually the same as cetiridine, but some of its effects are more remarkable than cetiridine.

Bepotastine significantly downmodulates CXCL10, CCL17 and IL-1α. CD54 expression is also suppressed by this antihistamine.

Olopatadine is unique in the preferential inhibition of the release of tachykinins such as substance P from sensory nerves (55). Moreover, this antihistamine downmodulates

the antigen-presenting ability of LC, and as a result, systemic administration of olopatadine suppresses CHS responses (56). Its effect on the keratinocyte chemokine production is detectable as those on substance P and LC, and olopatadine significantly suppresses the production of CXCL10 (56). The potential of olopatadine to suppress CCL17 production was observed in the peripheral blood of atopic patients administered with this antihistamine (57) and in LC.

Taken together these findings, antihistamines may serve as keratinocyte chemokine downmodulator with variations in their intensities. Their chemokine inhibitory properties seem to be clinically relevant. In light of the mechanisms of the CHS, the antihistamine-induced suppression of proinflammatory cytokines, IL-1α, TNF-α and granulocyte/macrophage colony-stimulating factor (GM-CSF), downmodulates LC maturation, resulting in depressed sensitization of the CHS. Inhibition of T cell migration to the epidermis by antihistamines may also occur, depending on their effect on Th1 or Th2 cells. The depressed production of Th1 and Th2 chemokines may lead to the delayed-type and late-phase reactions of the CHS, respectively. In addition, the suppression of CD54 expression on keratinocytes may inhibit T-cell adherence to the epidermis during the development of CHS. Epinastine has a potential to exert a therapeutic effect on Th1-mediated skin disorders, as it depresses pruritus in patients with not only AD (58) but also Th1-mediated psoriasis (59). Accordingly, it was approved by the ministry in Japan as a drug for psoriasis as well as eczematous diseases. Likewise, another Th1 chemokine inhibitor olopatadine has an approval for the treatment of psoriasis. On the other hand, fexofenadine is applicable for AD but not psoriasis because of its moiety for the Th2 chemokine inhibitor.

Regarding the mechanism by which the antihistmines exert their suppressive effects on keratinocyte cytokine/chemokine production by keratinocytes, a novel concept of H1 receptor function has been proposed. H1 receptors are G-protein-coupled receptors, and their inactive and active conformations co-exist in equilibrium. The activation level of the receptors in the absence of histamine is their 'constitutive activity' (60). In this scenario, histamine acts as an agonist and shifts the equilibrium towards the activated state. Antihistamines classified previously as antagonists function as either inverse agonists or neutral antagonists. Inverse agonists combine with and stabilize the inactive conformation of the receptor to shift the equilibrium towards the inactive state. Thus, they may downregulate constitutive receptor activity, even in the absence of histamine. Neutral antagonists combine equally with both conformations of the receptor, do not affect basal receptor activity, but do interfere with agonist binding. All H1 antihistamines examined to date are inverse agonists (60), including desloratadine, cetiridine, epinastine, loratadine and fexofenadine (61,62). In this concept, the term 'H1 receptor antagonists' is a misnomer. The observation that H1 receptors modulate nuclear factor-kappaB (NF-kB) activation (63) supports this receptor-dependent mechanism underlying anti-inflammatory actions of H1 antihistamines, including suppression of cytokines/chemokines and inhibition of CD54 expression. As basal activation of NF-kB through the H1 receptor is important for allergic inflammation, and antihistamines have no effect on NF-kB activity in the absence of the H1 receptor, it is likely that the suppressive effects of antihistaimines on the cytokine/chemokine production is mediated by the H1 receptor. In our experimental system, keratinocytes were stimulated by IFN-γ and TNF-α to produce chemokines. The fact that IFN-γ induces translocation of NF-κB in keratinocytes (64) further supports this concept.

Antibacterial drugs as a modulator of keratinocyte chemokine production

Some of the antibacterial agents have been known to serve as immunomodulators. Among them, macrolide antibacterial agents are the representative immunomodulatory drugs and well known to inhibit cytokine production by various cells (65–67). Erythromycin-derived 15-membered ring macrolides are structurally modified to permit unusually enhanced intracellular accumulation and have various modulatory bioactivities to immunocompetent cells that are involved in allergy and inflammation. Roxithromycin (RXM) (68), azithromycin (67,69) and clarithromycin (67) have been well studied and used for inflammatory conditions such as diffuse panbronchiolitis.

As summarized in Table 3, RXM modifies cutaneous immunity. RXM suppresses the functions of LC by downmodulating the expression of MHC class II and co-stimulatory molecules and the production of cytokines such as IL-1β (68), suggesting the potency of RXM to depress allergic contact dermatitis. Keratinocytes are another target of macrolides, as RXM downregulates the IFN-γ-enhanced expression of MHC class II molecules and the production of IL-1α and TNF-α and inhibits the superantigen-presenting function of keratinocytes (70). In accordance with these experimental findings, RXM exerts beneficial therapeutic effects on various inflammatory or immunologic skin disorders, including psoriasis, pustulosis palmaris et plantaris, AD, eosinophilic pustular folliculitis and prurigo pigmentosa (71–73).

RXM also downmodulates keratinocyte production of chemokines and T cell expression of chemokine receptors, suggesting its immunoregulatory capacity in the epidermal

milieu (11). In our study, RXM significantly suppressed the production/expression of Th2 chemokines CCL22 and CCL17 in keratinocytes, but the production of CXCL10 was not affected. The expression level of Th2 chemokine receptor CCR4 was decreased by RXM, whereas the expression of Th1 chemokine receptor CXCR3 was unchanged. Thus, the chemokine production and receptor expression in Th2 cells are preferentially downmodulated by RXM as compared with those in the Th1 cells. The optimal dose of RXM to suppress CCR4 expression and CCL22/CCL17 production ranges from 1 to 10 μM. As the concentration of RXM in the skin of individuals orally given 300 mg of RXM is approximately 13 μM (69), this optimal dose is considered to be meaningful in clinical settings. It is possible that RXM blocks the step of signalling common to these pathways in which NF-κB and other transcriptional factors are involved (66,74). In the production of CCL17 by HaCaT cells, it has been clarified that RXM suppresses the production through the inhibition of p38 and NF-κB, independent of IκB degradation (75).

Although a recent study (21) has suggested that CCL17 and CCL22 are derived mainly from LC, the differentiated roles of keratinocytes and LC for Th1 and Th2 chemokine production is still controversial. Therefore, the production of these Th2 chemokines by keratinocytes and their inhibition by RXM may be still potentially important. RXM inhibition of Th2 chemokine production in LC is an issue to be clarified in future.

The selective modulation of Th2 cells by RXM is informative for the clinical usage of this drug. A considerable number of diseases are known to be caused or mediated by Th2 cells. AD is a representative disorder, in which circulating and skin-infiltrating Th2 cells play an essential role in the pathogenesis. Others include subacute prurigo, eosinophilic pustular folliculitis, Wells' syndrome and angiolymphoid hyperplasia with eosinophilia or Kimura's disease. In some of these diseases, good therapeutic responses to RXM have already been reported or personally experienced. On the other hand, as for psoriasis, the mechanisms underlying the effectiveness involve improvement of T cell recruitment (71) and neutrophil activity (73).

Besides macrolides, nadifloxacin, an antiacne quinolone antimicrobial agent, is known to inhibit the production of proinflammatory cytokines by peripheral blood mononuclear cells and keratinocytes (76,77). Nadifloxacin suppresses the antigen-presenting function of LC for T cells. The ability of MHC class II$^+$ keratinocytes to present a superantigen to T cells is also suppressed by preincubation of keratinocytes with nadifloxacin. These functional reductions in LC and keratinocytes, together with the reduction in cytokine production by peripheral lymphocytes provide a possibility for nadifloxacin to inhibit chemokine production by keratinocytes.

Antifungal drugs as a modulator of keratinocyte chemokine production

While evidence for the role of innate immunity in fungal infection has been growing, another line of studies has suggested that antimycotic drugs are effective for some of the inflammatory skin diseases. For example, griseofulvin improves lichen planus (78), and ketoconazole is beneficial for AD (79) and seborrheic dermatitis (80). It has been shown that 0.5% of ketoconazole has a stronger anti-inflammatory capacity than 1% hydrocortisone (81). In this action, ketoconazole suppresses the production of 5-hydroxyeicosatetraenoic acid and leukotriene B4 without affecting cyclooxygenase or 12-lipoxygenase (82). Inversely, certain immunosuppressive drugs may have antifungal effects (83).

In human keratinocytes, it has been reported that ketoconazole and terbinafine hydrochloride suppress TNF-α-induced CCL27, CCL2 and CCL5 secretion and mRNA expression (84) as shown in Table 3. However, such inhibition was not found in HaCaT 17.5 keratinocytes by northern blot analysis (85).

β-glucans are a constituent of the cell wall of fungi including dermatophytes and can stimulate keratinocytes to produce proinflammatory cytokines and chemokines. As dermatophytes reside in the stratum corneum of the epidermis, it is a scenario in superficial dermatophytosis that the fungi stimulate keratinocytes to secrete chemokines attracting inflammatory cells. In this concept, we stimulated cultured keratinocytes with β-glucan or trichophytin from *Trichophyton* (*T.*) *rubrum* or *T. mentagrophytes*, and chemokines and cytokines in the culture supernatants were quantified. The production of CXCL8 and IL-1α was significantly enhanced by β-glucan or trichophytin. The increase of IL-8 was especially remarkable, and CXCL9 or CCL22 was not enhanced. Thus, CXCL8 was the most greatly enhanced chemokine/cytokine by the fungal elements, as has been reported previously (86). In this *in vitro* system, we added liranaftate, a Japanese representative thiocarbamate antifungal agent (87). β-glucan-augmented production of CXCL8 was profoundly suppressed by the addition of liranaftate to the culture in a dose-dependent manner. Liranaftate also depressed the trichophytin-promoted CXCL8 production significantly but at a lesser degree. Although the mechanism underlying the antifungal inhibition of CXCL8 remains unelucidated, the suppressed production of 5-hydroxyeicosatetraenoic acid, leukotriene B4 (82), CCL27, CCL2 and CCL5 (84) as well as CXCL8 provides an implication that antifungals finally can directly or indirectly inhibit NF-κB.

In dermatophytosis, fungi exist in the horny layer and cannot invade the lower part of the epidermis. When keratinocytes come in contact with fungi or their elements, a

strong inflammatory reaction seems to be evoked by the produced proinflammatory cytokine IL-1α and neutrophil chemoattractant CXCL8. In macrophages, it has been demonstrated that β-glucan is recognized by the Toll-like receptor (TLR) 2 and dectin-1 (88). It is likely that keratinocytes also recognize β-glucan through certain TLR or possibly dectin-1, leading to cutaneous inflammation as a consequence of the innate immune reactions.

In the cases of tinea pedis or tinea corporis, treatment of the associated inflammation is occasionally necessary for clinical improvement in addition to antifungal therapies. Antifungal agents possessing an anti-inflammatory property may be ideal in the treatment of tinea pedis. Evaluation of the anti-inflammatory action of antifungal drugs might provide valuable information. To assess this potential, the ability of a given agent to inhibit CXCL8 production is considered useful. CXCL8 is the key chemokine for neutrophils. Clinically, the antifungal drugs with CXCL8-decreasing activity may reduce infiltration of neutrophils in the skin and their invasion into the epidermis. Such drugs may improve unpleasant symptoms such as pustules and erosions in patients with tinea. On the other hand, however, the recruitment of neutrophils is an important defense mechanism against infection, and the inhibition of neutrophil infiltration by antifungals might prolong the fungal infection. The clinical benefit seems to be expressed as the sum of these beneficial and adverse effects.

Future perspectives

Drugs generally have various side effects. Provided that the effects are beneficial, not adverse, they might further improve conditions. Skin is exposed to internally or externally given drugs. It is likely that the chemokine production by keratinocytes is prone to be modulated by the given drugs. As keratinocyte-derived chemokines control the infiltration of T cells and polymorphonuclear leucocytes involved in the pathogenesis of cutaneous inflammatory diseases, the chemokine modulation resulting from antihistamines, antibiotics and antifungals is considered to substantially alleviate disease activity in patients administered with these drugs. The chemokine modulatory moiety may provide important information and therefore should be investigated in drugs used for inflammatory or infectious skin diseases.

References

1 Xu H, Bjarnason B, Elmets C A. Sensitization *versus* elicitation in allergic contact dermatitis: potential differences at cellular and molecular levels. Am J Contact Dermat 2000: **11**: 228–234.

2 Pastore S, Mascia F, Mariotti F, Dattilo C, Girolomoni G. Chemokine networks in inflammatory skin diseases. Eur J Dermatol 2004: **14**: 203–208.

3 Homey B, Steinhoff M, Ruzicka T, Leung D Y. Cytokines and chemokines orchestrate atopic skin inflammation. J Allergy Clin Immunol 2006: **118**: 178–189.

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

5 Boorsma D M, Flier J, Sampat S, *et al.* Chemokine IP-10 expression in cultured human keratinocytes. Arch Dermatol Res 1998: **290**: 335–341.

6 Xiao T, Kagami S, Saeki H, *et al.* Both IL-4 and IL-13 inhibit the TNF-α and IFN-γ enhanced MDC production in a human keratinocyte cell line, HaCaT cells. J Dermatol Sci 2003: **31**: 111–117.

7 Albanesi C, Scarponi C, Sebastiani S, *et al.* IL-4 enhances keratinocyte expression of CXCR3 agonistic chemokines. J Immunol 2000: **165**: 1395–1402.

8 Hino R, Shimauchi T, Tokura Y. Treatment with IFN-γ increases serum levels of Th1 chemokines and decreases those of Th2 chemokines in patients with mycosis fungoides. J Dermatol Sci 2005: **38**: 189–195.

9 Benoit S, Toksoy A, Brocker E B, Gillitzer R, Goebeler M. Treatment of recalcitrant pustular psoriasis with infliximab: effective reduction of chemokine expression. Br J Dermatol 2004: **150**: 1009–1012.

10 Hino R, Kobayashi M, Mori T, *et al.* Inhibition of T helper 2 chemokine production by narrowband ultraviolet B in cultured keratinocytes. Br J Dermatol 2007: **156**: 830–837.

11 Kobayashi M, Shimauchi T, Hino R, Tokura Y. Roxithromycin downmodulates Th2 chemokine production by keratinocytes and chemokine receptor expression on Th2 cells: its dual inhibitory effects on the ligands and the receptors. Cell Immunol 2004: **228**: 27–33.

12 Rossi D, Zlotnik A. The biology of chemokines and their receptors. Annu Rev Immunol 2000: **18**: 217–242.

13 Tokuriki A, Seo N, Ito T, Kumakiri M, Takigawa M, Tokura Y. Dominant expression of CXCR3 is associated with induced expression of IP-10 at hapten-challenged sites of murine contact hypersensitivity: a possible role for interferon-γ-producing $CD8^+$ T cells in IP-10 expression. J Dermatol Sci 2002: **28**: 234–241.

14 Schroder J M, Reich K, Kabashima K, *et al.* Who is really in control of skin immunity under physiological circumstances – lymphocytes, dendritic cells or keratinocytes? Exp Dermatol 2006: **15**: 913–929.

15 Flier J, Boorsma D M, van Beek PJ, *et al.* Differential expression of CXCR3 targeting chemokines CXCL10, CXCL9, and CXCL11 in different types of skin inflammation. J Pathol 2001: **194**: 398–405.

16 Rottman J B, Smith T L, Ganley K G, Kikuchi T, Krueger J G. Potential role of the chemokine receptors CXCR3, CCR4, and the integrin alphaEbeta7 in the pathogenesis of psoriasis vulgaris. Lab Invest 2001: **81**: 335–347.

17 Fukuoka M, Ogino Y, Sato H, *et al.* RANTES expression in psoriatic skin, and regulation of RANTES and IL-8 production in cultured epidermal keratinocytes by active vitamin D3 (tacalcitol). Br J Dermatol 1998: **138**: 63–70.

18 Albanesi C, Scarponi C, Sebastiani S, *et al.* A cytokine-to-chemokine axis between T lymphocytes and keratinocytes can favor Th1 cell accumulation in chronic inflammatory skin diseases. J Leuk Biol 2001: **70**: 617–623.

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 Xiao T, Fujita H, Saeki H, *et al.* Thymus and activation-regulated chemokine (TARC/CCL17) produced by mouse epidermal Langerhans cells is upregulated by TNF-α and IL-4 and downregulated by IFN-γ. Cytokine 2003: **23**: 126–132.

21 Mitsui H, Watanabe T, Saeki H, *et al.* Differential expression and function of Toll-like receptors in Langerhans cells: comparison with splenic dendritic cells. J Invest Dermatol 2004: **122**: 95–102.

22 Annels N E, Da Costa C E, Prins F A, Willemze A, Hogendoorn P C, Egeler R M. Aberrant chemokine receptor expression and chemokine production by Langerhans cells underlies the pathogenesis of Langerhans cell histiocytosis. J Exp Med 2003: **197**: 1385–1390.

23 Sallusto F, Lenig D, Mackay C R, Lanzavecchia A. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. J Exp Med 1998: **187**: 875–883.

24 Kagami S, Saeki H, Komine M, *et al.* CCL28 production in HaCaT cells was mediated by different signal pathways from CCL27. Exp Dermatol 2006: **15**: 95–100.

25 Tsuda T, Tohyama M, Yamasaki K, *et al.* Lack of evidence for TARC/CCL17 production by normal human keratinocytes *in vitro*. J Dermatol Sci 2003: **31**: 37–42.

26 Moed H, Boorsma D M, Tensen C P, *et al.* Increased CCL27-CCR10 expression in allergic contact dermatitis: implications for local skin immunity. J Pathol 2004: **204**: 39–46.

27 Sebastiani S, Albanesi C, Nasorri F, *et al.* Nickel-specific $CD4^+$ and $CD8^+$ T cells display distinct migratory responses to chemokines produced during allergic contact dermatitis. J Invest Dermatol 2002: **118**: 1052–1058.

28 Homey B Chemokines and inflammatory skin diseases. In: Advances in Dermatology, **Vol. 21**. New York: Mosby Inc., 2005: 251–277.

29 Wang B, Feliciani C, Freed I, Cai Q, Sauder D N. Insights into molecular mechanisms of contact hypersensitivity gained from gene knockout studies. J Leuk Biol 2001: **70**: 185–191.

30 Kissenpfennig A, Henri S, Dubois B, *et al.* Dynamics and function of Langerhans cells *in vivo*: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. Immunity 2005: **22**: 643–654.

31 Kaplan D H, Jenison M C, Saeland S, *et al.* Epidermal langerhans cell-deficient mice develop enhanced contact hypersensitivity. Immunity 2005: **23**: 611–620.

32 Barker J N W N, Mitra R S, Griffiths C E M, Dixit V M, Nickoloff B J. Keratinocytes as initiators of inflammation. Lancet 1991: **337**: 211–214.

33 Enk A H, Katz S I. Early molecular events in the induction phase of contact sensitivity. Proc Natl Acad Sci USA 1992: **89**: 1389–1402.

34 Gautam S, Battisto J, Major J A, Armstrong D, Stoler M, Hamilton T A. Chemokine expression in trinitrochlorobenzene-mediated contact hypersensitivity. J Leuk Biol 1994: **55**: 452–460.

35 Tensen C P, Flier J, Rampersad S S, *et al.* Genomic organization, sequence and transcriptional regulation of the human CXCL 11 gene. Biochem Biophys Acta 1999: **1446**: 167–172.

36 Flier J, Boorsma D M, Bruynzeel D P, *et al.* The CXCR3 activating chemokines IP-10, Mig, and IP-9 are expressed in allergic but not in irritant patch test reactions. J Invest Dermatol 1999: **113**: 574–578.

37 Kaplan G, Luster A D, Hancock G, Cohn Z A. The expression of a γ interferon-induced protein (IP-10) in delayed immune responses in human skin. J Exp Med 1987: **166**: 1098–1108.

38 Tensen C P, Vermeer M H, van der Stoop P M, *et al.* Epidermal interferon-γ inducible protein-10 (IP-10) and monokine induced by gamma-interferon (Mig) but not IL-8 mRNA expression is associated with epidermotropism in cutaneous T cell lymphomas. J Invest Dermatol 1998: **111**: 222–226.

39 Wang B, Fujisawa H, Zhuang L, *et al.* CD4+ Th1 and CD8+ type 1 cytotoxic T cells both play a crucial role in the full development of contact hypersensitivity. J Immunol 2000: **165**: 6783–6790.

40 Xu H, Dilulio N A, Fairchild R L. T cell populations primed by hapten sensitization in contact sensitivity are distinguished by polarized patterns of cytokine production: interferon gamma-producing (Tc1) effector CD8+ T cells and interleukin (Il) 4/Il-10-producing (Th2) negative regulatory CD4+ T cells. J Exp Med 1996: **183**: 1001–1012.

41 Dubois B, Chapat L, Goubier A, Papiernik M, Nicolas J F, Kaiserlian D. Innate CD4+ CD25+ regulatory T cells are required for oral tolerance and inhibition of CD8+ T cells mediating skin inflammation. Blood 2003: **102**: 3295–3301.

42 Imai S, Atarashi K, Ikesue K, Akiyama K, Tokura Y. Establishment of murine model of allergic photocontact dermatitis to ketoprofen and characterization of pathogenic T cells. J Dermatol Sci 2006: **41**: 127–136.

43 Suzuki K, Yamazaki S, Tokura Y. Expression of T-cell cytokines in challenged skin of murine allergic contact photosensitivity: low responsiveness is associated with induction of Th2 cytokines. J Dermatol Sci 2000: **23**: 138–144.

44 Amon U, Gibbs B F, Buss G, Nitschke M. *In vitro* investigations with the histamine H1 receptor antagonist, epinastine (WAL 801 CL), on isolated human allergic effector cells. Inflamm Res 2000: **49**: 112–116.

45 Kabashima K, Shiraishi N, Sugita K, *et al.*CXCL12-CXCR4 engagement is required for migration of cutaneous dendritic cells. Am J Pathol 2007: **171**: 1249–1257.

46 Kawashima M, Tango T, Noguchi T, Inagi M, Nakagawa H, Harada S. Addition of fexofenadine to a topical corticosteroid reduces the pruritus associated with atopic dermatitis in a 1-week randomized, multicentre, double-blind, placebo-controlled, parallel-group study. Br J Dermatol 2003: **148**: 1212–1221.

47 Ito J, Asano K, Tryka E, *et al.* Suppressive effects of co-stimulatory molecule expressions on mouse splenocytes by anti-allergic agents *in vitro*. Mediators Inflamm 2000: **9**: 69–75.

48 Matsukura M, Yajima A, Yamazaki F, *et al.* Epinastine inhibits eosinophil chemotaxis and adhesion molecules in atopic dermatitis. Skin Pharmacol Appl Skin Physiol 2003: **16**: 405–410.

49 Vignola A M, Crampette L, Mondain M, *et al.* Inhibitory activity of loratadine and descarboethoxyloratadine on expression of ICAM-1 and HLA-DR by nasal epithelial cells. Allergy 1995: **50**: 200–203.

50 Albanesi C, Pastore S, Fanales-Belasio E, Girolomoni G. Cetirizine and hydrocortisone differentially regulate ICAM-1 expression and chemokine release in cultured human keratinocytes. Clin Exp Allergy 1998: **28**: 101–109.

51 Izu K, Tokura Y. The various effects of four H1-antagonists on serum substance P levels in patients with atopic dermatitis. J Dermatol 2005: **32**: 776–781.

52 Kobayashi M, Kabashima K, Tokura Y. Inhibitory effects of epinastine on chemokine production and MHC class II/CD54 expression in keratinocytes. J Dermatol Sci 2007: **45**: 144–146.

53 Kobayashi M, Tokura Y. Preferential downmodulation of certain chemokines by fexofenadine in human keratinocytes. J Dermatol Sci 2005: **38**: 67–69.

54 Bruno G, Andreozzi P, Graf U, *et al.* Cetirizine, a second-generation H1 antagonist, modulates Rantes and MCP-1 levels in allergic rhinitis. Int J Immunopathol Pharmacol 2002: **15**: 113–118.

55 Ikemura T, Okarmura K, Sasaki Y, Ishi H, Ohmori K. KW-4679-induced inhibition of tachykininergic contraction in the guinea-pig bronchi by prejunctional inhibition of peripheral sensory nerves. Br J Pharmacol 1996: **118**: 1565–1573.

56 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.

57 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.
58 Tasaka K. Epinastine: an update of its pharmacology, metabolism, clinical efficacy and tolerability in the treatment of allergic diseases. Drugs Today 2000: **36**: 735–757.
59 Masutani M, Ueda H, Takeuchi M, *et al.* Clinical study of WAL801CL on psoriasis vulgaris with pruritus. J Clin Ther Med 1992: **8** (Suppl.1): 87–95.
60 Leurs R, Church M K, Taglialatela M. H1-antihistamines: inverse agonism, anti-inflammatory actions and cardiac effects. Clin Exp Allergy 2002: **32**: 489–498.
61 Bakker R A, Wieland K, Timmerman H, Leurs R. Constitutive activity of the histamine H(1) receptor reveals inverse agonism of histamine H(1) receptor antagonists. Eur J Pharmacol 2000: **387**: R5–7.
62 Wu R L, Anthes J C, Kreutner W, Harris A G, West R E. Desloratadine inhibits constitutive and histamine-stimulated nuclear factor-kappaB activity consistent with inverse agonism at the histamine H1 receptor. Int Arch Allergy Immunol 2004: **135**: 313–318.
63 Bakker R A, Schoonus S B, Smit M J, Timmerman H, Leurs R. Histamine H(1)-receptor activation of nuclear factor-kappa B: roles for G beta gamma- and G alpha(q/11)-subunits in constitutive and agonist-mediated signaling. Mol Pharmacol 2001: **60**: 1133–1142.
64 Kanda N, Shimizu T, Tada Y, Watanabe S. IL-18 enhances IFN-γ-induced production of CXCL9, CXCL10, and CXCL11 in human keratinocytes. Eur J Immunol 2007: **37**: 338–350.
65 Shinkai M, Foster G H, Rubin B K. Macrolide antibiotics modulate ERK phosphorylation and IL-8 and GM-CSF production by human bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol 2006: **290**: L75–L85.
66 Abeyama K, Kawahara K, Iino S, *et al.* Antibiotic cyclic AMP signaling by 'primed' leukocytes confers anti-inflammatory cytoprotection. J Leuk Biol 2003: **74**: 908–915.
67 Sugiyama K, Shirai R, Mukae H, *et al.* Differing effects of clarithromycin and azithromycin on cytokine production by murine dendritic cells. Clin Exp Immunol 2007: **147**: 540–546.
68 Ohshima A, Tokura Y, Wakita H, Furukawa F, Takigawa M. Roxithromycin down-modulates antigen-presenting and interleukin-1 β-producing abilities of murine Langerhans cells. J Dermatol Sci 1998: **17**: 214–222.
69 Tsai W C, Rodriguez M L, Young K S, *et al.* Azithromycin blocks neutrophil recruitment in *Pseudomonas* endobronchial infection. Am J Respir Crit Care Med 2004: **170**: 1331–1339.
70 Wakita H, Tokura Y, Furukawa F, Takigawa M. The macrolide antibiotic, roxithromycin suppresses IFN-γ-mediated immunological functions of cultured normal human keratinocytes. Biol Pharm Bull 1996: **19**: 224–227.
71 Ohshima A, Takigawa M, Tokura Y. $CD8^+$ cell changes in psoriasis associated with roxithromycin-induced clinical improvement. Eur J Dermatol 2001: **11**: 410–415.
72 Komine M, Tamaki K. An open trial of oral macrolide treatment for psoriasis vulgaris. J Dermatol 2000: **27**: 508–512.
73 Tokura Y, Sugaya K, Kageyama H, *et al.* Inhibitory effect of roxithromycin on the myeloperoxidase activity of peripheral polymorphonuclear leukocytes: its evaluation in patients with psoriasis and pustulosis palmaris et plantaris. Jpn J Inflammation 1995: **15**: 461–465.
74 Ohmori Y, Schreiber R D, Hamilton T A. Synergy between interferon-γ and tumor necrosis factor-α in transcriptional activation is mediated by cooperation between signal transducer and activator of transcription 1 and nuclear factor κB. J Biol Chem 1997: **272**: 14899–14907.
75 Komine M, Kakinuma T, Kagami S, Hanakawa Y, Hashimoto K, Tamaki. Mechanism of thymus- and activation-regulated chemokine (TARC)/CCL17 production and its modulation by roxithromycin. J Invest Dermatol 2005: **125**: 491–498.
76 Kuwahara K, Kitazawa T, Kitagaki H, Tsukamoto T, Kikuchi M. Nadifloxacin, an antiacne quinolone antimicrobial, inhibits the production of proinflammatory cytokines by human peripheral blood mononuclear cells and normal human keratinocytes. J Dermatol Sci 2005: **38**: 47–55.
77 Murata K, Sugita K, Kobayashi M, Kabashima K, Tokura Y. Nadifloxacin downmodulates antigen-presenting functions of epidermal Langerhans cells and keratinocytes. J Dermatol Sci 2006: **42**: 91–99.
78 Sehgal V N, Abraham G J, Malik G B. Griseofulvin therapy in lichen planus. A double-blind controlled trial. Br J Dermatol 1972: **87**: 383–385.
79 Back O, Scheynius A, Johansson S G. Ketoconazole in atopic dermatitis: therapeutic response is correlated with decrease in serum IgE. Arch Dermatol Res 1995: **287**: 448–451.
80 Ford G P, Farr P M, Ive F A, Shuster S. The response of seborrheic dermatitis to ketoconazole. Br J Dermatol 1984: **111**: 603–607.
81 Van Cutsem J, Van Gerven F, Cauwenbergh G, Odds F, Janssen P A. The antiinflammatory effects of ketoconazole. A comparative study with hydrocortisone acetate in a model using living and killed Staphylococcus aureus on the skin of guinea pigs. J Am Acad Dermatol 1991: **25**: 257–261.
82 Beetens J R, Loots W, Somers Y, Coene M C, De Clerck F. Ketoconazole inhibits the biosynthesis of leukotrienes *in vitro* and *in vivo*. Biochem Pharmacol 1986: **35**: 883–891.
83 Sugita T, Tajima M, Ito T, Saito M, Tsuboi R, Nishikawa A. Antifungal activities of tacrolimus and azole agents against the eleven currently accepted *Malassezia* species. J Clin Microbiol 2005: **43**: 2824–2829.
84 Kanda N, Watanabe S. Suppressive effects of antimycotics on tumor necrosis factor-α-induced CCL27, CCL2, and CCL5 production in human keratinocytes. Biochem Pharmacol 2006: **72**: 463–473.
85 Friccius H, Pohla H, Adibzadeh M, Siegels-Hubenthal P, Schenk A, Pawelec G. The effects of the antifungal azole itraconazole, fluconazole, ketoconazole and miconazole on cytokine gene expression in human lymphoid cells. Int J Immunopharmacol 1992: **14**: 791–799.
86 Koga T, Ishizaki H, Matsumoto T, Toshitani S. Enhanced release of interleukin-8 from human epidermal keratinocytes in response to stimulation with trichophytin *in vitro*. Acta Derm Venereol 1996: **76**: 399–400.
87 Oku Y, Sakuma K, Yokoyama K, Miyaji M. Fungal activity of liranaftate against *Trichophyton rubrum*. Jpn J Med Mycol 2002: **43**: 181–187.
88 Gantner B N, Simmons R M, Canavera S J, Akira S, Underhill D M. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. J Exp Med 2003: **197**: 1107–1117.

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Biotin-deficiency up-regulates TNF-α production in vivo and in vitro

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Abstract. Biotin, a water-soluble vitamin of the B complex, functions as a cofactor of carboxylases that catalyze indispensable cellular metabolism. It was reported that the concentrations of biotin were significantly lower in sera of patients with chronic inflammatory diseases. However, the biological roles of biotin in inflammatory responses are unclear. In this study, we investigated the effects of biotin-deficiency on tumor necrosis factor (TNF)-α production in vivo and in vitro. Mice were fed a basal diet or a biotin-deficient diet for 8 weeks. After intravenous administration of lipopolysaccharide (LPS), serum TNF-α levels in biotin-deficient mice were significantly higher than those in biotin-sufficient mice. A murine macrophage-like cell line, J774.1, was cultured in biotin-sufficient or biotin-deficient medium. Biotin-deficient J774.1 cells produced TNF-α significantly higher than biotin-sufficient J774.1 cells in response to LPS and even without LPS stimulation. Moreover, biotin-supplementation inhibited TNF-α production of biotin-deficient cells. Addition of cyclic guanosine 5′-monophosphate (cGMP) significantly decreased TNF-α production of the biotin-deficient cells, indicating that up-regulation of TNF-α production was regulated by cGMP-dependent signaling pathways. In conclusion, these results suggest that biotin is critically involved in inflammatory diseases via the regulation of TNF-α production in vivo and in vitro.

Key words. biotin, macrophage, TNF-α, cGMP

1 Introduction

Biotin is a water-soluble vitamin of the B complex found in all organisms [1]. Biotin functions as a cofactor of four carboxylases: pyruvate carboxylase, acetyl-CoA carboxylase, propionyl-CoA carboxylase, and 3-methylcrotonyl-CoA carboxylase [1]. These enzymes catalyze the metabolism of glucose, amino acids, and fatty acids. In addition to this classical function as a cofactor of carboxylases, biotin is involved in various cellular events. Biotin regulates the mRNA expression of holocarboxylase synthetase and biotin-dependent carboxylases via cGMP-dependent pathway [2]. Moreover, some transcription factors, such as Sp1, Sp3, and NF-κB, were regulated by biotin, and the biotinylation of histones in human cells was also reported [3, 4]. These reports clearly indicate that biotin regulates the various cellular events at the transcriptional levels.

Biotin-deficiency causes alopecia and scaly erythematous dermatitis [5]. Moreover, it was reported that serum biotin levels are significantly lower in atopic dermatitis patients than in healthy subjects [6]. Biotin has a therapeutic effect on pustulosis palmaris et plantaris, a type of chronic dermatitis which is restricted to the palms and soles [7]. These reports suggest that biotin-deficiency is involved in inflammatory diseases. However, few reports are available on the biological roles of biotin in inflammatory responses.

In this study, we investigate the effects of biotin-deficiency on the production of TNF-α in vivo and in vitro.

2 Experimental procedures

2.1 Mice

Female BALB/c mice (4 weeks old) received a basal diet (AIN-76) or a biotin-deficient AIN-76 diet. The Ethical Board for nonhuman species of the Tohoku University Graduate School of Medicine approved the experimental procedure followed in this study. Concentrations of biotin in serum were measured with ELISA [8].

2.2 Measurement of TNF-α

Concentrations of TNF-α were measured with a commercial ELISA kit.

2.3 Cells and cell culture

Murine macrophage-like J774.1 cells were grown in biotin-sufficient or biotin-deficient medium. The biotin-sufficient medium was RPMI 1640 containing *d*-biotin (0.2 mg/μg) supplemented with 10% FCS. The biotin-deficient medium was biotin free RPMI 1640 supplemented with 10% biotin-deficient FCS. Biotin in FCS was depleted with immobilized avidin-agarose. J774.1 cells were cultured with biotin-deficient medium for 4 weeks, and then further incubated in the medium without biotin (biotin-deficiency) or with biotin (biotin-supplementation) for 2 weeks. J774.1 cells were also cultured in biotin-sufficient medium for 6 weeks (biotin-sufficiency).

2.4 Data analysis

All of the experiments in this study were performed at least three times to confirm the reproducibility of the results. The data shown are representative results. Experi-

mental values are given as the mean ± SD of triplicate assays. Statistical analysis was performed with the unpaired *t*-test or one-way ANOVA using Dunnett's method, and $P < 0.05$ was considered significant.

3 Results

3.1 Augmentation of serum TNF-α levels in biotin-deficient mice injected with LPS

After 8 weeks of feeding with biotin-sufficient or biotin-deficient diets, the serum concentrations of biotin in biotin-deficient group were significantly ($P < 0.01$) lower than those in biotin-sufficient group. No clinical symptoms were detected in the biotin-deficient group, and no significant differences of body weights were detected between biotin-sufficient and biotin-deficient groups. A significant ($P < 0.01$) increase of the serum TNF-α level was induced 90 min after i.v. injection of LPS (2 μg/kg) (Fig. 1). In biotin-deficient group, the concentration of TNF-α was significantly ($P < 0.05$) higher than that in biotin-sufficient group. These results indicated that biotin-deficiency augments TNF-α production in vivo.

3.2 Augmentation of TNF-α production in biotin-deficient J774.1 cells

Next, we analyzed TNF-α production by biotin-sufficient and biotin-deficient J774.1 cells. As shown in Fig. 2, both types of cells were produced TNF-α in a dose-dependent manner with LPS stimulation. The concentration of TNF-α in the culture supernatants of biotin-deficient cells was significantly ($P < 0.01$) higher than that of biotin-sufficient cells even without LPS stimulation. These results clearly indicated that biotin-deficiency induces the augmentation of TNF-α production in vitro.

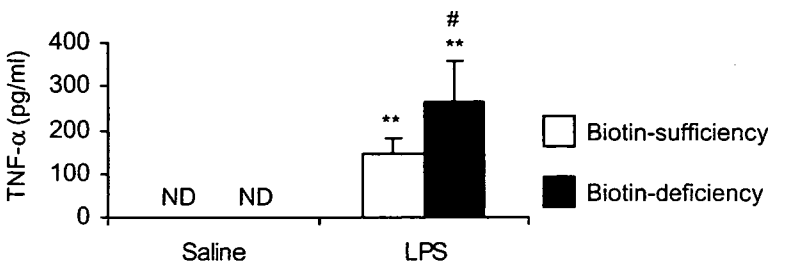

Fig. 1. Serum level of TNF-α in biotin-sufficient and biotin-deficient mice. Biotin-sufficient and biotin-deficient mice were challenged i.v. with LPS (2 μg/kg) or saline alone, and blood was taken at 90 min after injection. The results were expressed as mean ± SD for four mice. *ND*, Not detected. **, $P < 0.01$, compared with saline. #, $P < 0.05$, compared with biotin-sufficiency