Prostaglandin E_2–EP_3 signaling suppresses skin inflammation in murine contact hypersensitivity

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Background: Prostaglandin (PG) E_2 exerts a variety of actions through 4 G protein–coupled receptors designated as EP_1, EP_2, EP_3, and EP_4. We have reported that PGE_2 acts on EP_3 in airway epithelial cells and exerts anti-inflammatory actions in ovalbumin-induced murine allergic asthma. Although EP_3 is also expressed in skin and PGE_2 is produced abundantly during skin allergic inflammation, the role of PGE_2-EP_3 signaling in skin allergic inflammation remains unknown.

Objective: We sought to investigate whether PGE_2-EP_3 signaling exerts anti-inflammatory actions in skin allergic inflammation.

Methods: We used a murine contact hypersensitivity (CHS) model and examined the role of EP_3 by using an EP_3-selective agonist, ONO-AE-248 (AE248), and EP_3-deficient mice. The inflammation was evaluated by the thickness and histology of the hapten-challenged ear. Inflammation-associated changes in gene expression and effects of AE248 were examined by means of microarray analysis of the skin. Localization of EP_3 was examined by staining for β-galactosidase knocked in at the EP_3 locus in EP_3-deficient mice. EP_3 action was also examined in cultured keratinocytes.

Results: Administration of AE248 during the elicitation phase significantly suppressed CHS compared with that seen in vehicle-treated mice. Microarray analysis revealed that administration of AE248 inhibited the gene expression of neutrophil-recruiting chemokines, including CXCL1, at the elicitation site. X-gal staining in EP_3-deficient mice revealed EP_3 expression in keratinocytes, which was further confirmed by anti-EP_3 antibody in wild-type mice. In cultured keratinocytes AE248 suppressed CXCL1 production induced by TNF-α.

Conclusion: PGE_2-EP_3 signaling inhibits keratinocytes activation and exerts anti-inflammatory actions in murine CHS. (J Allergy Clin Immunol 2009;124:809-18.)

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Murine contact hypersensitivity (CHS) is widely used as a model for contact dermatitis, a common allergic skin disorder of human subjects. The CHS model is composed of 2 phases: the sensitization phase, in which skin dendritic cells take up antigens, migrate to regional lymph nodes, and stimulate T-cell activation and differentiation, and the elicitation phase, in which effector T cells evoke immune inflammation on exposure to antigens.[1] Although the elicitation reaction is known to be mediated by IFN-γ–producing T_H1 cells and T cytotoxic type 1 cells, it is suggested that initial neutrophil infiltration is required for subsequent recruitment of T cells and development of inflammation.[2,3] On exposure to antigens in the elicitation phase, keratinocytes produce neutrophil-recruiting chemokines, such as CXCL1 and CXCL2, as well as T cell–recruiting chemokines, such as CCL17 or CCL27, which contribute to neutrophil recruitment within 12 hours after elicitation and after T-cell infiltration, respectively.[3-5] At an inflammatory site, other than chemokines or cytokines, lipid mediators, such as prostanoids, are produced abundantly, which might regulate CHS responses.[6,7]

Prostanoids, including prostaglandin (PG) D_2, PGE_2, $PGF_{2\alpha}$, PGI_2 (prostacyclin), and thromboxane A_2, are oxygenated metabolites of arachidonic acid produced by sequential catalysis of COX and respective synthases. They are produced in large amounts during inflammation in response to various stimuli and exert a variety of actions, including inflammatory swelling, pain sensation, and fever generation. Prostanoids exert these actions by acting on a family of G protein–coupled receptors, which include PGD receptor, 4 subtypes of PGE receptor (EP_1, EP_2, EP_3, and EP_4), PGF receptor, PGI receptor, and thromboxane A receptor.[8] In addition, another receptor belonging to the chemokine receptor family, CRTH2, also responds to PGD_2. PGE_2 and PGD_2 are abundantly produced in the skin during the elicitation phase of CHS.[6,9] It has been shown that PGD_2 promotes neutrophil infiltration through CRTH2 and contributes to progression of inflammation during elicitation.[9] However, the role of PGE_2 in the elicitation phase has not been fully investigated. Furthermore, if the above action of the PGD_2-CRTH2 signaling is the only PG-mediated action involved in elicitation of a CHS response, nonsteroidal anti-inflammatory drugs (NSAIDs) that inhibit COX and suppress PG production would suppress or lessen allergic inflammation in the skin. However, NSAIDs are usually without significant effects on the inflammation of CHS, suggesting the presence of other PG receptor–mediated processes that suppress inflammation.

On the basis of this hypothesis, we have examined the action of PGE_2 in allergic skin inflammation. Among EPs, we focused on

Abbreviations used
AE248: ONO-AE-248
CHS: Contact hypersensitivity
DNFB: 2,4-Dinitrofluorobenzene
HE: Hematoxylin and eosin
LT: Leukotriene
NSAID: Nonsteroidal anti-inflammatory drug
PG: Prostaglandin
PMN: Polymorphonuclear leukocyte
WT: Wild-type

EP_3 because EP_3 is expressed abundantly in the skin[10,11] and mediates suppression of allergic inflammation in the murine allergic asthma model.[12] Although EP_3 has been reported to have both proinflammatory and anti-inflammatory roles in patients with acute skin inflammation,[13,14] the role of EP_3 signaling in allergic skin inflammation has not been investigated. Here we used an EP_3-selective agonist and EP_3-deficient *(Ptger3*$^{-/-}$*)* mice and examined whether PGE_2-EP_3 signaling has anti-inflammatory action during the elicitation phase of CHS.

METHODS

Materials

Female 8- to 12-week-old C57BL/6 mice (Japan SLC, Shizuoka, Japan) and mice lacking EP_3 that were backcrossed to a C57BL/6 background for more than 10 generations[15] were used. Mice were bred at the Institute of Laboratory Animals of Kyoto University on a 12-hour light/dark cycle under specific pathogen-free conditions. All experimental procedures were approved by the Committee on Animal Research of Kyoto University Faculty of Medicine. The EP agonists ONO-DI-004 (EP_1 agonist), ONO-AE1-259 (EP_2 agonist), ONO-AE-248 (AE248; EP_3 agonist), and ONO-AE1-329 (EP_4 agonist) were kindly provided by Ono Pharmaceutical Co (Osaka, Japan). The structures, ligand-binding affinities and selectivities, and pharmacokinetic properties of each EP agonist were described.[8] 2,4-Dinitrofluorobenzene (DNFB) was purchased from Nacalai Tesque (Kyoto, Japan). Indomethacin was purchased from Sigma (St Louis, Mo).

CHS experiment

CHS was induced as previously described.[16] Briefly, mice were shaved and painted on the abdomen with 25 μL of 0.5% DNFB in acetone/olive oil (4:1). Five days later, the mice were challenged by painting with 10 μL of 0.3% DNFB on both sides of the ear. Ear thickness was measured with a thickness gage (Teclok, Nagano, Japan) before and 24 hours after the challenge, and the difference was used as a parameter of ear swelling. AE248 was diluted with saline or acetone and administered either subcutaneously in the dorsal skin or topically applied to the ear 3 times a day (30 minutes before and 3 and 8 hours after the DNFB challenge, respectively) at indicated doses. For repeated DNFB application, mice were sensitized first by means of topical application of 20 μL of 0.15% DNFB to both ears and challenged with 20 μL of 0.15% DNFB on both ears once a week for 4 weeks. Vehicle (acetone) or indomethacin (0.2 mg/mL in acetone, 20 μL per ear) was applied 30 minutes before each challenge.

Bone marrow transplantation

Bone marrow cells were taken from femurs from wild-type (WT) or *Ptger3*$^{-/-}$ donor mice and transplanted to recipient WT green fluorescent protein transgenic mice (2×10^6 cells for each mouse from the tail vein) irradiated with 8 Gy. Four weeks after transplantation, more than 97% of whole blood cells were reconstituted with donor-derived cells, which was confirmed by analyzing the expression of green fluorescent protein–positive cells among blood cells with flow cytometry, and we used those mice for experiments.

Histology

Ears were isolated 24 hours after elicitation, fixed in 10% formalin, and embedded in paraffin. Sections of 7 μm in thickness were prepared and stained with hematoxylin and eosin (HE). The number of neutrophils per a ×40 field was determined in 4 randomly chosen fields, and the average counts were determined. For EP_3 localization, X-gal staining was performed as previously described.[12] The sections were then counterstained with HE or anti-keratin 5 antibody (R&D Systems, Minneapolis, Minn). For staining of EP_3, the rabbit polyclonal antibody reactive with murine EP_3 (Cayman, Ann Arbor, Mich) was used as previously described.[17]

Real-time RT-PCR

Total RNA was obtained from keratinocytes of murine ear skin by using the RNeasy Mini Kit (Qiagen, Hilden, Germany). Complementary DNA was synthesized with Superscript III (Invitrogen, Carlsbad, Calif). The amount of mRNA for CXCL1 and glyceraldehyde-3-phosphate dehydrogenase was quantified by means of real-time RT-PCR with the LightCycler 2.0 (Roche Diagnostic, Foster City, Calif). The primer sequences of glyceraldehyde-3-phosphate dehydrogenase were previously described.[18] Primers used for CXCL1 were 5′-GCC TAT CGC CAA TGA GC-3′ (forward) and 5′-TGG ACA ATT TTC TGA ACC AAG-3′ (reverse). Data were analyzed by using LightCycler Software Version 4.0.

Keratinocyte culture and ELISA

Normal human epidermal keratinocytes were obtained from Kurabo (Okayama, Japan) and cultured in Humedia KG2 medium (Kurabo). Cells in the third passage were seeded in triplicate at 5×10^4 cells/well onto 24-well plates in 0.5 mL of Humedia KB2 and cultured for 24 hours. The cells were washed, incubated with 10 μmol/L AE248 for 15 minutes, and then incubated with 10 ng/mL TNF-α in the continued presence of AE248 in Humedia KB2 containing 1 μmol/L indomethacin for 6 hours. The supernatant was collected, and the amount of CXCL1 was determined by means of ELISA (R&D Systems).

DNA microarray analysis

Total RNA was prepared from DNFB-challenged ears by using TRIzol reagent (Invitrogen) and purified by using the RNeasy Mini Kit (Qiagen), and 3.5 μg of purified RNA was used for microarray analysis with a Mouse Genome 430 2.0 Array (Affymetrix, Santa Clara, Calif), according to the manufacturer's protocol. Data were analyzed by using Statistical Algorithm with the Affymetrix GeneChip Expression Analysis software (Microarray Suite 5.0). All microarray data are deposited in Gene Expression Omnibus (GEO).

Statistics

Data were expressed as means ± SEMs, and statistical analyses were performed by means of ANOVA or the Student *t* test, as appropriate. A *P* value of less than .05 was considered statistically significant.

RESULTS

Effect of EP_3 agonist on the elicitation phase of CHS

We first examined whether stimulation of EP_3 had an anti-inflammatory effect on CHS. To investigate this, we administered an EP_3 agonist CAE248, 100 μg/kg subcutaneously 3 times a day during the elicitation phase. This dose of AE248 exerts a significant effect *in vivo*.[12] The DNFB challenge caused ear swelling in both vehicle-treated and AE248-treated mice. However, the mice treated with AE248 showed significant reductions in swelling

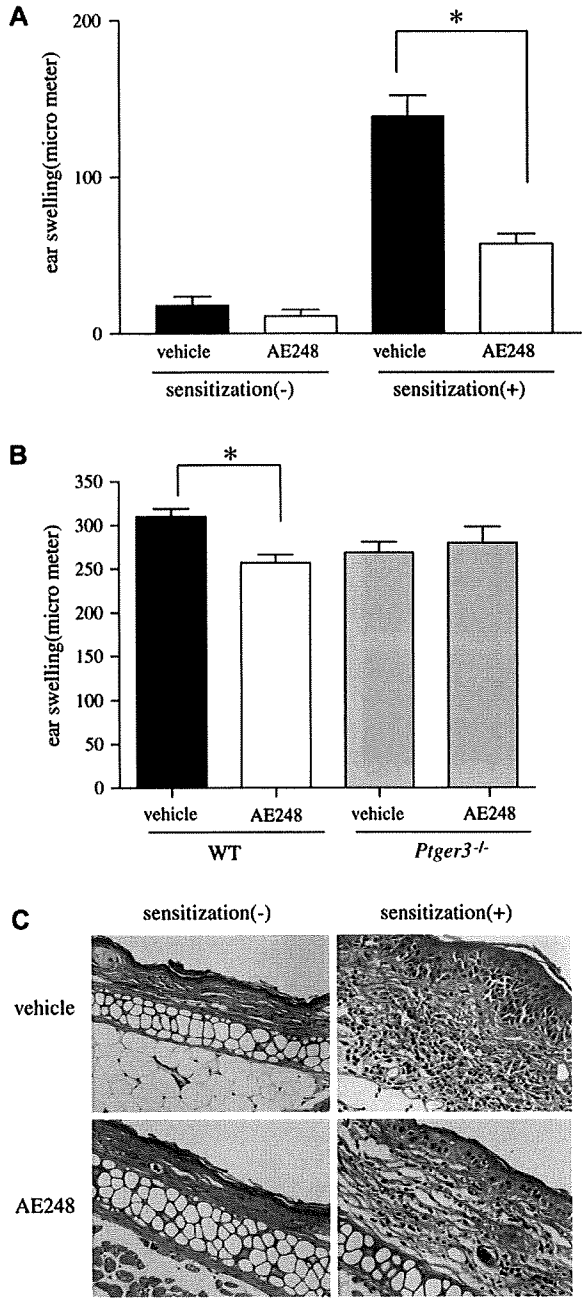

FIG 1. Suppressive effects of EP_3 agonist on the ear-swelling response in mice with CHS. **A** and **B**, Results are expressed as means ± SEMs (n = 5 in both groups). Data are representative of 3 experiments. **C**, HE staining of control- and DNFB-challenged ears treated with either vehicle or EP_3 agonist. Representative samples of each group are shown.

compared with that seen in the vehicle-treated mice 24 hours after elicitation (Fig 1, *A*). This suppressive effect of AE248 was completely absent in *Ptger3*$^{-/-}$ mice (Fig 1, *B*), suggesting that the effect was elicited through the EP_3 receptor. Histology of the ear from sensitized mice showed edema and marked inflammatory cell infiltration in the dermis 24 hours after elicitation (Fig 1, *C*). Consistently, the extent of the edema and inflammatory cell infiltration was markedly reduced in the AE248-treated mice compared with that seen in the vehicle-treated mice. These findings together demonstrate that EP_3 stimulation in the elicitation phase elicits suppressive effect on CHS.

We next examined the localization of EP_3 in the normal murine ear. X-gal staining was performed in *Ptger3*$^{-/-}$ mice, in which the β-galactosidase gene was knocked in at the EP_3 gene locus. Positive signals were detected mostly in the basal layer of epidermis in the skin of control mice (Fig 2, *A*). Similar signals were also observed in the ears of mice after elicitation, whereas little signals were detected in the cells infiltrating the dermis (data not shown). These findings suggest that the main cell species expressing EP_3 in the skin is keratinocytes and that they express it constitutively. To examine the EP_3 expression in keratinocytes of the basal layer, we costained for keratin 5, a specific marker of basal keratinocytes, and found that signals for keratin 5 colocalized with those of the X-gal staining (Fig 2, *A*). EP_3 expression in keratinocytes was confirmed by means of immunohistochemical analysis with anti-EP_3 antibody in WT mice (Fig 2, *B*). These results, together with our finding that little X-gal staining was detected in lymph nodes (data not shown), suggest a possibility that AE248 acts on keratinocytes and not on immune cells to exert its anti-inflammatory actions. Therefore we next examined the effects of topical application of AE248 to the ear in CHS. AE248 was dissolved in acetone and topically applied to the ear 3 times in the elicitation phase. This topical application of AE248 showed significant dose-dependent suppression of ear swelling in CHS 24 hours after elicitation (Fig 2, *C*), whereas that of agonists specific to other EP subtypes was without effect (Fig 2, *D*). Administration of AE248 showed a suppressive effect 24, 48, and 72 hours after elicitation, suggesting that the effect of AE248 did not induce just the delay in the development of inflammation (Fig 2, *E*). To confirm that the effect of AE248 was not caused by immune cells, we made bone marrow chimera in which stromal cells, such as keratinocytes, express EP_3, whereas bone marrow–derived cells do not express EP_3, as described in the Methods section. A suppressive effect of AE248 on ear swelling in CHS of the bone marrow chimera was detected (Fig 2, *F*), which supports our hypothesis that AE248 acts on EP_3 in keratinocytes to exert an anti-inflammatory effect.

Reduced expression of genes related to inflammatory cell infiltration caused by topical treatment with AE248

Various inflammation-related genes, including those for chemokines, are upregulated during the elicitation phase of CHS.[5] We therefore compared gene expression between vehicle-treated control mice and mice treated with AE248 to examine the role of EP_3 in this process. We first examined the time course of gene expression during the elicitation phase in our model. Ears challenged with DNFB were isolated at 1, 3, 6, 12, and 24 hours after elicitation for microarray analysis by using an Affymetrix Mouse Genome 430 2.0 GeneChip that contains 45,101 genes. We screened for gene expression, which exhibited a more than 2-fold increase at any given time during the elicitation phase over basal expression at 0 hours (Table I). Among the genes with increased expression, we focused on chemokine genes. At 1 hour after challenge, 130 genes were detected as genes showing a more than 2-fold increase in expression, and none of chemokine genes was among those genes. At 3 hours, 263 genes were upregulated, and 4 kinds of chemokines were included in this group. The analysis similarly picked up 408 genes with 5 kinds of chemokine genes at 6 hours, 655 genes with 8 kinds of chemokine genes at 12 hours, and 902 genes with 14 kinds of chemokine

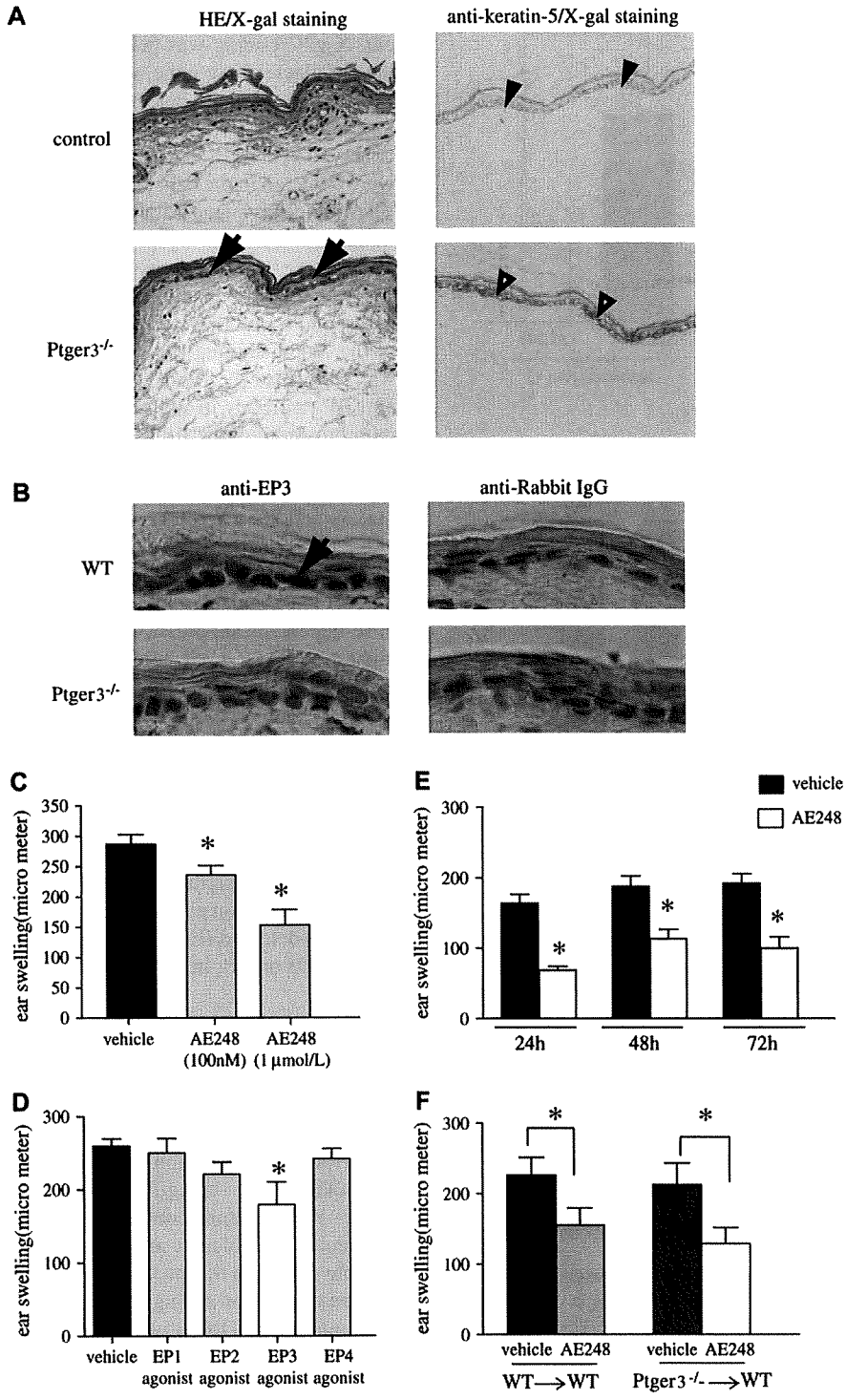

FIG 2. Localization of EP_3 receptors and effect of topical application of EP_3 agonist on mice with CHS. **A**, Histochemical staining for EP_3 (X-gal) counterstained with HE or anti–keratin 5 antibody. *Arrows*, Positive signaling (blue); *black arrowheads*, positive staining of keratin 5; *white arrowheads*, colocalization of positive signals in X-gal and anti–keratin 5 staining. **B**, Immunohistologic analysis for EP_3. The *arrow* indicates positive signals. **C-E**, Suppressive effects of topical administration of EP_3 agonist and effects of various EP agonists (1 μmol/L) on murine CHS (n = 5 per group [Fig 2, *C*] and n = 4 per group [Fig 2, *D* and *E*]). Data are representative of 2 experiments. **F**, Effect of AE248 on murine CHS of bone marrow chimera (n = 13-15 per group). Results are a combination of 3 independent experiments.

TABLE I. Time course of chemokine-related genes with expressions upregulated more than 2-fold in the elicitation phase

Time (h)	No. of genes	Gene title	Gene symbol	Probe set ID	Change ratio
1	130				
3	263	Chemokine (C-X-C motif) ligand 1	*Cxcl1*	1419209_at	2.2
		Chemokine (C-X-C motif) ligand 1	*Cxcl1*	1441855_x_at	1
		Chemokine (C-X-C motif) ligand 2	*Cxcl2*	1449984_at	1.3
		Chemokine (C-C motif) ligand 7	*Ccl7*	1421228_at	1.3
		Chemokine (C-C motif) ligand 20	*Ccl20*	1422029_at	1.2
6	408	Chemokine (C-C motif) ligand 17	*Ccl17*	1419413_at	2.6
		Chemokine (C-C motif) ligand 20	*Ccl20*	1422029_at	2.6
		Chemokine (C-X-C motif) ligand 1	*Cxcl1*	1419209_at	2.5
		Chemokine (C-X-C motif) ligand 1	*Cxcl1*	1457644_s_at	1
		Chemokine (C-X-C motif) ligand 16	*Cxcl16*	1449195_s_at	1.2
		Chemokine (C-C motif) ligand 9	*Ccl9*	1417936_at	1
12	655	Chemokine (C-X-C motif) ligand 9	*Cxcl9*	1456907_at	4.5
		Chemokine (C-X-C motif) ligand 9	*Cxcl9*	1418652_at	2.9
		Chemokine (C-X-C motif) ligand 1	*Cxcl1*	1419209_at	3.8
		Chemokine (C-X-C motif) ligand 1	*Cxcl1*	1441855_x_at	2.2
		Chemokine (C-X-C motif) ligand 1	*Cxcl1*	1457644_s_at	2.1
		Chemokine (C-X-C motif) ligand 16	*Cxcl16*	1449195_s_at	1.4
		Chemokine (C-C motif) ligand 9	*Ccl9*	1417936_at	1.2
		Chemokine (C-X-C motif) ligand 10	*Cxcl10*	1418930_at	2.8
		Chemokine (C-C motif) ligand 20	*Ccl20*	1422029_at	2.4
		Chemokine (C-X-C motif) ligand 2	*Cxcl2*	1449984_at	1.7
		Chemokine (C-C motif) ligand 2	*Ccl2*	1420380_at	1.2
24	902	Chemokine (C-X-C motif) ligand 9	*Cxcl9*	1418652_at	5.2
		Chemokine (C-X-C motif) ligand 9	*Cxcl9*	1456907_at	5.1
		Chemokine (C-X-C motif) ligand 10	*Cxcl10*	1418930_at	4
		Chemokine (C-C motif) ligand 17	*Ccl17*	1419413_at	2.5
		Chemokine (C-C motif) receptor 1	*Ccr1*	1419609_at	2.4
		Chemokine (C-X-C motif) ligand 1	*Cxcl1*	1419209_at	2.2
		Chemokine (C-C motif) ligand 12	*Ccl12*	1419282_at	2.1
		Chemokine (C-C motif) ligand 2	*Ccl2*	1420380_at	2
		Chemokine (C-X-C motif) ligand 2	*Cxcl2*	1449984_at	1.8
		Chemokine (C-C motif) ligand 9	*Ccl9*	1448898_at	1.4
		Chemokine (C-C motif) ligand 9	*Ccl9*	1417936_at	1.2
		Chemokine (C-C motif) receptor 2	*Ccr2*	1421186_at	1.3
		Chemokine (C-C motif) ligand 7	*Ccl7*	1421228_at	1.3
		Chemokine (C-C motif) ligand 19	*Ccl19*	1449277_at	1.3
		Chemokine (C-X-C motif) ligand 16	*Cxcl16*	1449195_s_at	1.2
		Chemokine (C-C motif) ligand 8	*Ccl8*	1419684_at	1

genes at 24 hours. Because these results show the most prominent change in gene expression at 24 hours after elicitation, we chose this time and compared gene expression in the ears of vehicle-treated mice with that in ears of AE248-treated mice (Fig 3). Among the 902 genes, the signal intensity of 178 genes was significantly decreased in the AE248-treated group compared with that seen in the vehicle-treated group (cluster A), and signal intensity of 183 genes was significantly increased in the AE248-treated group (cluster B). The signal intensity of the other 541 genes was not significantly different between the groups. As for chemokine genes of significant signal intensity, cluster A includes genes for 3 chemokines: CXCL1, CXCL9, and CXCL16. Among them, expression of CXCL1 was most strongly suppressed by the AE248 treatment; the signal intensity decreased to 52% compared with the control intensity (Table II), and the average intensity of the other 2 chemokine genes, CXCL9 and CXCL16, decreased to 62% and 69%, respectively, compared with the control intensity. On the other hand, 4 chemokine genes, CCL19, CCL9, CCL8, and CCL12, were detected in cluster B. The average signal intensity of CCL19, CCL9, CCL8, and CCL12 in the AE248-treated group was 129%, 268%, 368%, and 404%, respectively, of that in the vehicle-treated group. Thus the treatment with AE248 did not decrease all of the chemokines upregulated 24 hours after the challenge. However, it nonetheless suppressed the inflammatory response in CHS, indicating that suppression of the expression of the chemokine genes in cluster A (ie, CXCL1, CXCL9, and CXCL16) has an important role in the CHS response. CXCL1 binds to CXCR2 and is one of the strong neutrophil-attracting chemokines. CXCL9 recruits T_H1 cells by binding cell-surface CXCR3 and contributes to the development of CHS.[5] CXCL16 is known to bind and activate the chemokine receptor CXCR6, which is expressed on T cells and natural killer T cells.[19] Given the critical role of CXCL1 and neutrophils in the development of CHS,[2,3] we further examined the roles of CXCL1 and neutrophils in CHS. We performed real-time RT-PCR analysis and confirmed that the expression of CXCL1 mRNA was

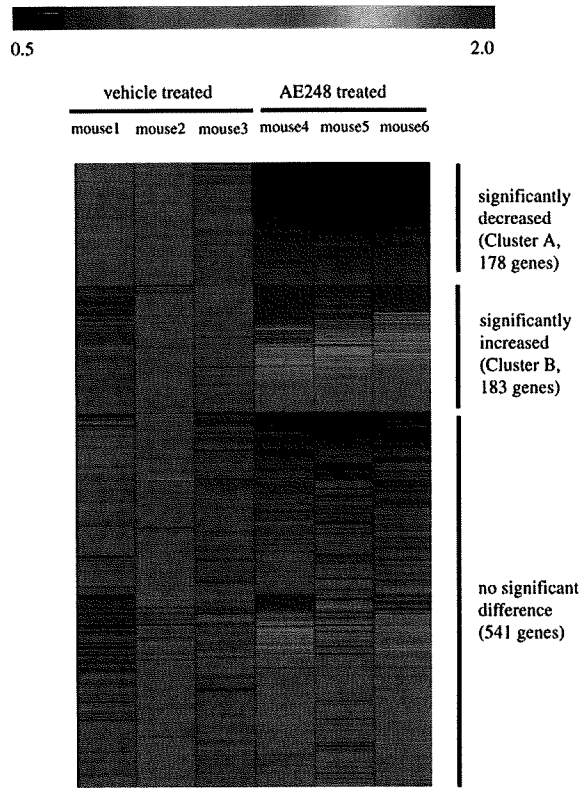

FIG 3. Microarray analysis for the effect of AE248 on gene expressions. Genes with a signal intensity that increased more than 2-fold in 24 hours at elicitation from baseline are selected (total of 902 genes). The signal intensity of each gene in the AE248-treated group (n = 3) was compared with the average signal intensity of each gene in the vehicle-treated group (n = 3), and the change ratio was indicated by means of color gradation.

significantly decreased in the AE248-treated group compared with that seen in the vehicle-treated group (data not shown). CXCL1 is produced mainly by keratinocytes in the elicitation phase of CHS. To confirm whether differences in gene expression result from differences in cell composition rather than from changes in gene expression in keratinocytes, we next examined the effect of AE248 on the mRNA expression of CXCL1 in keratinocytes from murine ear skin. We purified keratinocytes as shown in the Methods section of this article's Online Repository at www.jacionline.org. We then examined CXCL1 mRNA expression in purified keratinocytes and found that the AE248-treated group had significantly lower CXCL1 mRNA expression compared with that seen in the vehicle-treated group (Fig 4, *A*). We further examined the effect of AE248 on the production of CXCL1 by cultured keratinocytes *in vitro*. We found that keratinocytes activated with TNF-α produced a significant amount of CXCL1, and the administration of AE248 significantly suppressed the CXCL1 production (Fig 4, *B*). We next compared neutrophil (polymorphonuclear leukocytes [PMNs]) infiltration in the ears of the vehicle-treated and AE248-treated mice, as shown in the Methods section. We detected a number of PMNs in the dermis of the ear 24 hours after elicitation, whereas only a few cells were detected in the dermis of control animals. We also detected PMNs in the ears of the AE248-treated mice. However, the number of PMNs was significantly reduced in the AE248-treated mice compared with that seen in the vehicle-treated group (Fig 4, *C*). Because the average signal intensity of CXCL2 in microarray analysis, which is another important chemokine for neutrophil recruitment, was 44% of the vehicle-treated group in the AE248-treated group, we examined the CXCL2 mRNA expression in purified keratinocytes. We found that the AE248-treated group had significantly lower CXCL2 mRNA expression compared with that seen in the vehicle-treated group (see Fig E1 in this article's Online Repository at www.jacionline.org). These results combined together suggest a possibility that EP_3 exerts its anti-inflammatory effect by acting directly on keratinocytes and inhibiting neutrophil infiltration through downregulation of neutrophil-recruiting chemokines.

Involvement of endogenous PGE_2-EP_3 signaling in the development of CHS

We next examined the involvement of endogenous PG signaling in the development of CHS by applying indomethacin topically to the elicitation site. Because the effects of indomethacin were hard to detect in the usual CHS protocol (data not shown), we adopted the repetitive challenge model of CHS (repeated-challenge CHS) and examined the effect of indomethacin on the model as in the Methods section. Mice treated with indomethacin showed significantly increased ear swelling compared with the vehicle-treated mice (Fig 5, *A*), suggesting that endogenous PG produced locally in the challenged skin plays a suppressive role in inflammation of repeated-challenge CHS. We next examined the involvement of PGE_2-EP_3 signaling in this process by subjecting *Ptger3*$^{-/-}$ mice to repeated-challenge CHS. Similarly to the indomethacin-treated mice, *Ptger3*$^{-/-}$ mice exhibited significantly increased ear swelling compared with that of WT mice, and this enhancement continued 72 hours after elicitation (Fig 5, *B*). HE staining showed increased inflammatory cell infiltration in *Ptger3*$^{-/-}$ mice compared with that seen in WT mice (Fig 5, *C*). These results suggest that PGE_2-EP_3 signaling functions endogenously to negatively modulate the development of repeated-challenge CHS.

DISCUSSION

In the present study we have made the following findings. First, systemic administration of AE248, an EP_3 agonist, during the elicitation phase, can suppress inflammation in mice with CHS. Data of X-gal staining and immunohistochemical analysis demonstrated predominant EP_3 expression in keratinocytes, and topical application of AE248 to the ear skin resulted in suppression of CHS. Microarray analyses revealed that administration of AE248 modulates CHS-induced gene expression in the lesional skin either way; a decrease and an increase were demonstrated in clusters A and B, respectively. Among the chemokine genes regulated by AE248, CXCL1 was the most strongly suppressed. Consistently, AE248 suppressed CXCL1 production by TNF-α–activated keratinocytes *in vitro*. Finally, local treatment with indomethacin or the loss of EP_3 exacerbated inflammatory response to the repeated-challenge CHS. These results suggest that endogenous PGE_2 acts on EP_3 in keratinocytes *in situ* in the skin to modulate the extent of inflammation of CHS and that stimulation of PGE_2-EP_3 signaling with exogenously added agonist can control allergic inflammation in the skin.

The importance of keratinocytes in inflammatory skin diseases, such as contact dermatitis,[2,4] atopic dermatitis,[20] and psoriasis,[21,22]

TABLE II. Top 20 genes with signal intensities that were significantly decreased in AE248-treated mice compared with those in vehicle-treated mice

Gene title	Gene symbol	Probe set ID	Signal intensity		Percentage of AE248/vehicle
			Vehicle	AE248	
Solute carrier family 26, member 4	*Slc26a4*	1419725_at	2431	664.6	27.3
Matrix metallopeptidase 10	*Mmp10*	1420450_at	3371	1152	34.2
PG-endoperoxide Synthase 2	*Ptgs2*	1417262_at	3377	1345	39.8
SH2 domain containing 5	*Sh2d5*	1436100_at	2729	1118	41
Small proline-rich protein 2I	*Sprr2i*	1422963_at	13751	6136	44.6
Serine (or cysteine) peptidase inhibitor, clade E, member 1	*Serpine1*	1419149_at	801.6	359	44.8
Interferon-activated gene 202B	*Ifi202b*	1457666_s_at	15766	7122	45.1
Solute carrier family 29 (nucleoside transporters), member 2	*Slc29a2*	1447748_x_at	359.4	166.6	46.4
Fos-like antigen 1	*Fosl1*	1417487_at	3827	1785	46.6
IL-6	*Il6*	1450297_at	2010	955.7	47.6
Heparin-binding EGF-like growth factor	*Hbegf*	1418349_at	3090	1490	48.2
Cardiotrophin-like cytokine factor 1	*Clcf1*	1437270_a_at	1047	530.9	50.7
Chemokine (C-X-C motif) ligand 1	*Cxcl1*	1419209_at	4085	2131	52.2
Nucleolar complex–associated 2 homolog *(S cerevisiae)*	*Noc2l*	1424323_at	1638	867.1	52.9
RIKEN cDNA 2310002A05 gene /// hypothetical protein LOC630971	*2310002A05Rik /// LOC630971*	1456248_at	25273	13462	53.3
Similar to late cornified envelope protein	*LOC545548*	1456001_at	19223	10243	53.3
Sulfiredoxin 1 homolog *(S cerevisiae)*	*Srxn1*	1426875_s_at	4664	2525	54.1
Defensin β 3	*Defb3*	1421806_at	17218	9328	54.2
Serine (or cysteine) peptidase inhibitor, clade A (α-1 antiproteinase, antitrypsin), member 9	*Serpina9*	1429285_at	1362	750.5	55.1
RIKEN cDNA 2310007F04 gene	*2310007F04Rik*	1429641_x_at	9952	5505	55.3

The average signal intensity of each gene was compared between vehicle-treated mice and AE248-treated mice.
EGF, Epidermal growth factor.

has now received much attention. In CHS keratinocytes produce various chemokines and regulate inflammatory cell infiltration. For example, it was previously shown that blockade of CCL27, which is produced from keratinocytes and attracts memory T cells to the skin, reduced T-cell infiltration and led to the suppression of CHS,[4] and inhibition of CCL8 produced from keratinocytes suppressed CHS.[22] In this study we found that EP_3 is expressed in keratinocytes and that administration of EP_3 agonist suppresses CXCL1 mRNA expression and its production in keratinocytes. CXCL1 is a strong attractant of neutrophils and is produced mainly by keratinocytes in the elicitation phase of CHS.[2,3] Recently, it has been reported that infiltration of neutrophils is required for the development of inflammation in CHS.[2] Depletion of neutrophils in hapten-challenged mice decreased the number of IFN-γ–producing T cells at elicitation sites, which resulted in the inhibition of CHS response, and injection of neutrophils into ears restored the CHS response.[2,3] Administration of anti-CXCL1 serum in the elicitation phase significantly inhibited the neutrophil infiltration in the challenged ear and suppressed CHS.[3] Furthermore, it has been reported that corticosteroids exert their anti-inflammatory effects in contact dermatitis mainly by targeting neutrophils and macrophages.[23] These findings are consistent with our results described above and suggest that one of the anti-inflammatory effects of AE248 is through EP_3-mediated downregulation of CXCL1

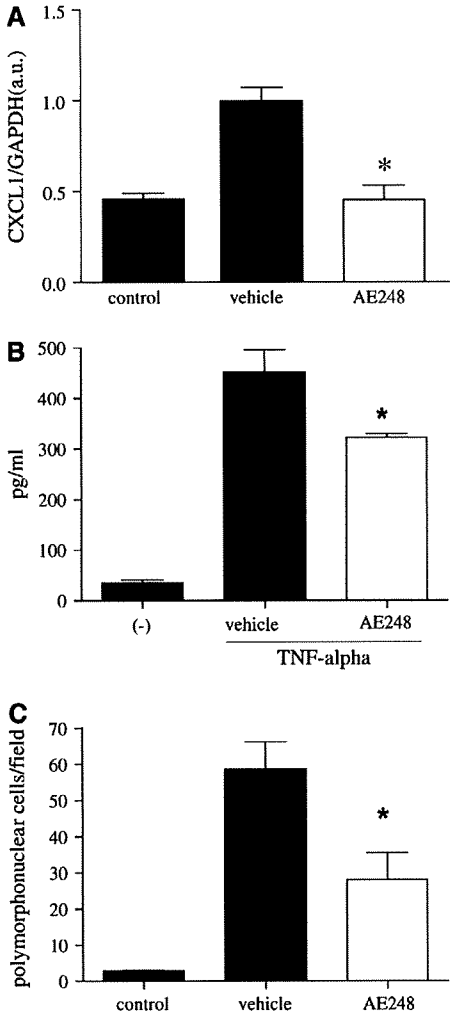

FIG 4. Effect of AE248 on CXCL1 production and neutrophil accumulation at the elicitation site. **A,** Real-time RT-PCR analysis on mRNA expression of CXCL1 in the keratinocytes in hapten-challenged ears of vehicle- or AE248-treated mice (n = 3 per group). **B,** ELISA analysis of CXCL1 production from cultured keratinocytes (n = 3 per group). Results are expressed as means ± SEMs and are representative of 3 independent experiments. **C,** Number of polymorphonuclear cells in the skin (n = 4 per group). *$P < .05$ versus the vehicle-treated group.

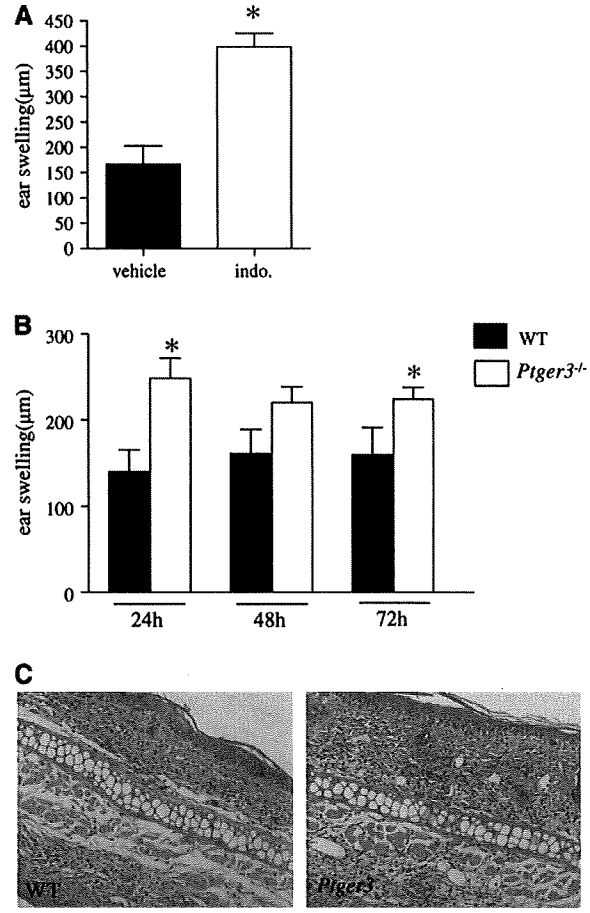

FIG 5. Enhanced inflammation of indomethacin *(indo)*-treated mice or *Ptger3*$^{-/-}$ mice in repeated-challenge CHS. **A,** Effect of topical administration of indomethacin on murine CHS (n = 4 per group). **B,** Increased ear swelling of *Ptger3*$^{-/-}$ mice in the CHS group (n = 4 per group). *$P < .05$ versus WT mice. Data are representative of 3 independent experiments. **C,** Representative HE staining of ear skin in WT and *Ptger3*$^{-/-}$ mice with repeated-challenge CHS at 24 hours after the fourth challenge.

production in keratinocytes and inhibition of neutrophil infiltration in the skin. On the other hand, our study showed that the EP_3 stimulation might have an effect on other chemokines opposite to that expected from the previous studies. For example, the expression of CCL8 was increased more than 3-fold in the AE248-treated group. In addition, we did not detect significant expression of CCL27 in our model. Given the finding that inhibition of CCL8 leads to suppression of inflammation, as described above, it is intriguing that EP_3 stimulation can reduce the inflammatory reaction in spite of such enhanced CCL8 gene expression.Unraveling this apparent discrepancy might help to reveal intricate relations among chemokines in inducing skin inflammation and define more correctly how EP_3 modulates their interaction. Taken together, chemokines from keratinocytes contribute much to the inflammation of CHS, and regulation of their production can be a useful strategy for the treatment of allergic dermatitis.

It should mentioned here that EP_3 is also expressed in mast cells in skin[24] and that mast cells can regulate the elicitation of CHS.[25,26] Indeed, expression of CXCL2 mRNA, which mast cells produce and was reported to promote neutrophil infiltration in elicitation sites, decreased to 44% of the control expression with EP_3 stimulation, although the difference was not statistically significant. However, we found that CXCL2 mRNA expression was significantly decreased in AE248-treated ears on purified keratinocytes. In addition, data of bone marrow chimera experiments indicate that most of the effect of AE248 was through stromal cells, suggesting that the anti-inflammatory effect of EP_3 derived from its action on keratinocytes.

EP_3 has 3 splice variants in the mouse, and 8 splice variants in human subjects,[27] among which at least 3 EP_3 variants are expressed in human keratinocytes.[10] They can couple Gs, Gi, and Gq and use cyclic AMP or Ca^{2+} as second messengers. EP_3 signaling can use ceramide as a second messenger in keratinocytes.[10] Our study does not clarify which signaling mechanism of EP_3 is responsible for its anti-inflammatory effect. The fact that a small dose of AE248 can induce anti-inflammatory effects is consistent with the Gi pathway because the cyclic AMP decrease mediated by Gi occurs at lower agonist

concentrations than other signaling. Indeed, the CB1 cannabinoid receptor that couples to Gi was recently reported to act on keratinocytes and suppress CHS inflammation.[28] However, this article reports that stimulation of CB1 inhibits CCL8 production from keratinocytes, which, as described, is opposite to our findings. These results indicate that multiple signaling pathways might function downstream of EP_3. It was shown recently that the Ca^{2+} signaling from EP_3 can inhibit nuclear factor κB activation in keratinocytes,[29] which is consistent with regulation of expression of CXCL1 mRNA by nuclear factor κB activity.[30] The precise molecular mechanisms underlying the anti-inflammatory effect of EP_3 remain to be elucidated.

Our results might also explain the anti-inflammatory effect of PGE_2 in skin inflammation and the adverse effect of NSAIDs in inflammatory skin diseases, such as psoriasis. Psoriasis is one of the inflammatory skin diseases, and constitutive activation of keratinocytes has been suggested as one of its causes.[21,22] Histologically, neutrophil infiltration in the epidermis is one of its characteristic features. Although PGE_2 is generally considered an inflammatory mediator by increasing vasodilation and edema formation, the anti-inflammatory effect of PGE_2 has been suggested in patients with psoriasis[31] or in animal models of neutrophil infiltration in skin.[32] On the other hand, administration of NSAIDs sometimes causes the exacerbation of psoriasis.[33,34] Thus far, the increase in levels of leukotrienes (LTs), such as LTB_4, a strong chemoattractant for neutrophils or T cells, has been suggested as one of its causative mechanisms.[33,34] Because arachidonic acid is used by both COX and lipoxygenase and NSAIDs block only COX activity, the use of NSAIDs might divert arachidonate metabolism to the lipoxygenase pathway, which leads to the increase of LTB_4. Such an argument was also made in aspirin-induced asthma. However, our study appears against such diversion mechanism. Alternatively, the PGE_2 pathway somehow modulates LT production. Our results suggest that one of the therapeutic effects of PGE_2 and an adverse effect of NSAIDs in skin inflammation is through modulation of PGE_2-EP_3 signaling.

In conclusion, stimulation of EP_3 signaling suppresses skin inflammation in CHS. Regulation of EP_3 signaling and keratinocyte function might be a novel approach for the treatment of skin inflammation, including allergy.

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Clinical implications: EP_3 in keratinocytes can be a target for the treatment of allergic skin inflammation.

REFERENCES

1. Grabbe S, Schwarz T. Immunoregulatory mechanisms involved in elicitation of allergic contact hypersensitivity. Immunol Today 1998;19:37-44.
2. Engeman T, Gorbachev AV, Kish DD, Fairchild RL. The intensity of neutrophil infiltration controls the number of antigen-primed CD8 T cells recruited into cutaneous antigen challenge sites. J Leukoc Biol 2004;76:941-9.
3. Dilulio NA, Engeman T, Armstrong D, Tannenbaum C, Hamilton TA, Fairchild RL. Groalpha-mediated recruitment of neutrophils is required for elicitation of contact hypersensitivity. Eur J Immunol 1999;29:3485-95.
4. Homey B, Alenius H, Muller A, Soto H, Bowman EP, Yuan W, et al. CCL27-CCR10 interactions regulate T cell-mediated skin inflammation. Nat Med 2002; 8:157-65.
5. Mitsui G, Mitsui K, Hirano T, Ohara O, Kato M, Niwano Y. Kinetic profiles of sequential gene expressions for chemokines in mice with contact hypersensitivity. Immunol Lett 2003;86:191-7.
6. Ruzicka T, Printz MP. Arachidonic acid metabolism in skin: experimental contact dermatitis in guinea pigs. Int Arch Allergy Appl Immunol 1982;69:347-52.
7. Eberhard J, Jepsen S, Albers HK, Acil Y. Quantitation of arachidonic acid metabolites in small tissue biopsies by reversed-phase high-performance liquid chromatography. Anal Biochem 2000;280:258-63.
8. Narumiya S, Sugimoto Y, Ushikubi F. Prostanoid receptors: structures, properties, and functions. Physiol Rev 1999;79:1193-226.
9. Takeshita K, Yamasaki T, Nagao K, Sugimoto H, Shichijo M, Gantner F, et al. CRTH2 is a prominent effector in contact hypersensitivity-induced neutrophil inflammation. Int Immunol 2004;16:947-59.
10. Konger RL, Brouxhon S, Partillo S, VanBuskirk J, Pentland AP. The EP3 receptor stimulates ceramide and diacylglycerol release and inhibits growth of primary keratinocytes. Exp Dermatol 2005;14:914-22.
11. Tober KL, Thomas-Ahner JM, Kusewitt DF, Oberyszyn TM. Effects of UVB on E prostanoid receptor expression in murine skin. J Invest Dermatol 2007;127: 214-21.
12. Kunikata T, Yamane H, Segi E, Matsuoka T, Sugimoto Y, Tanaka S, et al. Suppression of allergic inflammation by the prostaglandin E receptor subtype EP3. Nat Immunol 2005;6:524-31.
13. Ahluwalia A, Perretti M. Anti-inflammatory effect of prostanoids in mouse and rat skin: evidence for a role of EP3-receptors. J Pharmacol Exp Ther 1994;268: 1526-31.
14. Goulet JL, Pace AJ, Key ML, Byrum RS, Nguyen M, Tilley SL, et al. E-prostanoid-3 receptors mediate the proinflammatory actions of prostaglandin E2 in acute cutaneous inflammation. J Immunol 2004;173:1321-6.
15. Ushikubi F, Segi E, Sugimoto Y, Murata T, Matsuoka T, Kobayashi T, et al. Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. Nature 1998;395:281-4.
16. Kabashima K, Sakata D, Nagamachi M, Miyachi Y, Inaba K, Narumiya S. Prostaglandin E2-EP4 signaling initiates skin immune responses by promoting migration and maturation of Langerhans cells. Nat Med 2003;9:744-9.
17. Ueta M, Matsuoka T, Narumiya S, Kinoshita S, Tomimatsu H, Miyauchi Y, et al. Prostaglandin E receptor subtype EP3 in conjunctival epithelium regulates late-phase reaction of experimental allergic conjunctivitis. J Allergy Clin Immunol 2009;123:466-71.
18. Honda T, Segi-Nishida E, Miyachi Y, Narumiya S. Prostacyclin-IP signaling and prostaglandin E2-EP2/EP4 signaling both mediate joint inflammation in mouse collagen-induced arthritis. J Exp Med 2006;203:325-35.
19. Latta M, Mohan K, Issekutz TB. CXCR6 is expressed on T cells in both T helper type 1 (Th1) inflammation and allergen-induced Th2 lung inflammation but is only a weak mediator of chemotaxis. Immunology 2007;121:555-64.
20. Yoo J, Omori M, Gyarmati D, Zhou B, Aye T, Brewer A, et al. Spontaneous atopic dermatitis in mice expressing an inducible thymic stromal lymphopoietin transgene specifically in the skin. J Exp Med 2005;202:541-9.
21. Sano S, Chan KS, Carbajal S, Clifford J, Peavey M, Kiguchi K, et al. Stat3 links activated keratinocytes and immunocytes required for development of psoriasis in a novel transgenic mouse model. Nat Med 2005;11:43-9.
22. Zenz R, Eferl R, Kenner L, Florin L, Hummerich L, Mehic D, et al. Psoriasis-like skin disease and arthritis caused by inducible epidermal deletion of Jun proteins. Nature 2005;437:369-75.
23. Tuckermann JP, Kleiman A, Moriggl R, Spanbroek R, Neumann A, Illing A, et al. Macrophages and neutrophils are the targets for immune suppression by glucocorticoids in contact allergy. J Clin Invest 2007;117:1381-90.
24. Nguyen M, Solle M, Audoly LP, Tilley SL, Stock JL, McNeish JD, et al. Receptors and signaling mechanisms required for prostaglandin E2-mediated regulation of mast cell degranulation and IL-6 production. J Immunol 2002;169: 4586-93.
25. Grimbaldeston MA, Nakae S, Kalesnikoff J, Tsai M, Galli SJ. Mast cell-derived interleukin 10 limits skin pathology in contact dermatitis and chronic irradiation with ultraviolet B. Nat Immunol 2007;8:1095-104.
26. Biedermann T, Kneilling M, Mailhammer R, Maier K, Sander CA, Kollias G, et al. Mast cells control neutrophil recruitment during T cell-mediated delayed-type hypersensitivity reactions through tumor necrosis factor and macrophage inflammatory protein 2. J Exp Med 2000;192:1441-52.
27. Matsuoka T, Narumiya S. Prostaglandin receptor signaling in disease. Sci World J 2007;7:1329-47.
28. Karsak M, Gaffal E, Date R, Wang-Eckhardt L, Rehnelt J, Petrosino S, et al. Attenuation of allergic contact dermatitis through the endocannabinoid system. Science 2007;316:1494-7.
29. Kanda N, Mitsui H, Watanabe S. Prostaglandin E(2) suppresses CCL27 production through EP2 and EP3 receptors in human keratinocytes. J Allergy Clin Immunol 2004;114:1403-9.
30. Scortegagna M, Cataisson C, Martin RJ, Hicklin DJ, Schreiber RD, Yuspa SH, et al. HIF-1{alpha} regulates epithelial inflammation by cell autonomous

NF{kappa}B activation and paracrine stromal remodeling. Blood 2008;111: 3343-54.
31. Hebborn P, Jablonska S, Beutner EH, Langner A, Wolska H. Action of topically applied arachidonic acid on the skin of patients with psoriasis. Arch Dermatol 1988;124:387-91.
32. Ruzicka T, Burg G. Effects of chronic intracutaneous administration of arachidonic acid and its metabolites. Induction of leukocytoclastic vasculitis by leukotriene B4 and 12-hydroxyeicosatetraenoic acid and its prevention by prostaglandin E2. J Invest Dermatol 1987;88:120-3.
33. Lazarova AZ, Tsankov NK, Zlatkov NB. Psoriasis induced by topically applied indomethacin. Clin Exp Dermatol 1989;14:260-1.
34. Powles AV, Griffiths CE, Seifert MH, Fry L. Exacerbation of psoriasis by indomethacin. Br J Dermatol 1987;117:799-800.

METHODS

mRNA extraction of purified keratinocytes from murine ear skin

The hairs on murine ear skin were removed with depilatory cream. After removing hairs, the ears were split into dorsal and ventral halves, and the cartilage was removed. Then the skin was floated on 0.25% trypsin/EDTA for 30 minutes at 37°C and separated into epidermis and dermis. Single epidermal cell suspension (EC suspension) was done by means of vigorous trituration of the epidermal sheet. Because EC suspensions are mixtures of keratinocytes, Langerhans cells, and γδ T cells, we purified keratinocytes by removing Langerhans cells and γδ T cells from the EC suspension with the autoMACS system (Miltenyi Biotec, Bergisch Gladbach, Germany) using magnetic microbeads coated with anti-mouse CD45 antibody. The purity of keratinocytes is greater than 99%. Then we extracted RNA from keratinocytes with the RNeasy mini kit (Qiagen).

Real time RT-PCR primer

The primer sequences of CXCL2 were 5′-GCC TAT CGC CAA TGA GC-3′ (forward) and 5′-TGG ACA ATT TTC TGA ACC AAG-3′ (reverse).

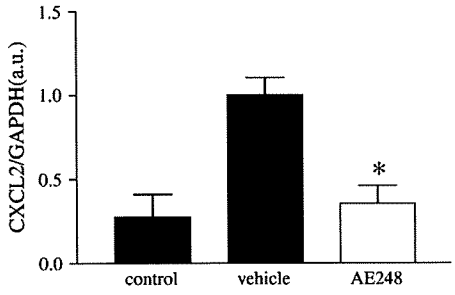

FIG E1. Real-time RT-PCR analysis on mRNA expression of CXCL2 in the purified keratinocytes in hapten-challenged ears of vehicle- or AE248-treated mice (n = 3 per group). Results are expressed as means ± SEMs. *$P < .05$ versus the vehicle-treated group. *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase.

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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

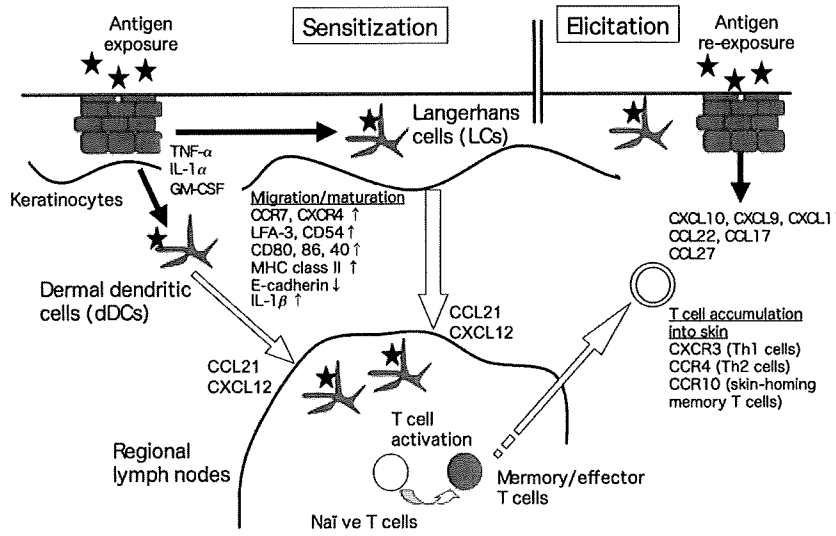

Figure 1. Schematic representation of mechanisms of contact hypersensitivity (CHS) and participation of keratinocyte chemokines. Sensitization phase of CHS: upon exposure to exogenous antigens, epidermal keratinocytes produce tumor necrosis factor (TNF)-α, interleukin (IL)-1α and GM-CSF, thereby inducing 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.